



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

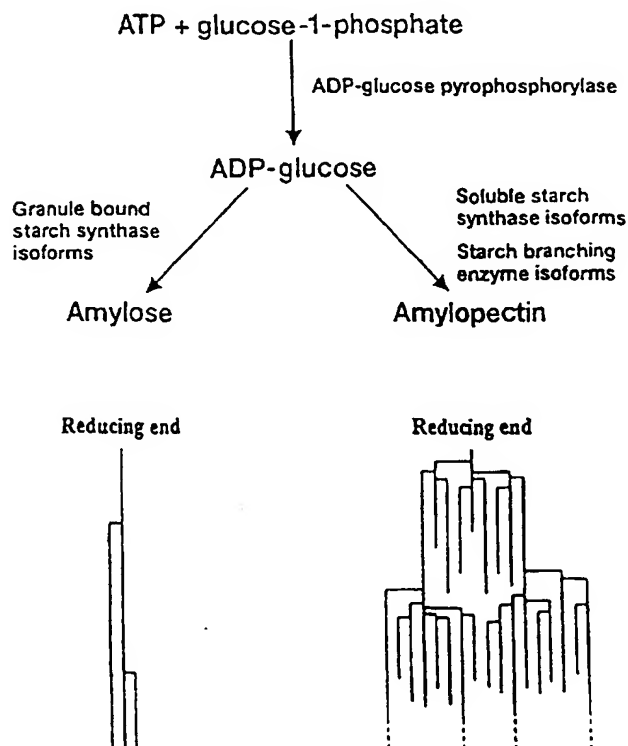
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/82, 9/10, 15/11, C08B 30/04		A1	(11) International Publication Number: WO 98/37213
			(43) International Publication Date: 27 August 1998 (27.08.98)
(21) International Application Number: PCT/IB98/00270		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 23 February 1998 (23.02.98)		Published With international search report.	
(30) Priority Data: 9703663.6 21 February 1997 (21.02.97) GB 9706060.2 24 March 1997 (24.03.97) GB			
(71) Applicant (for all designated States except US): DANISCO A/S [DK/DK]; Langebrogade 1, P.O. Box 17, DK-1001 Copenhagen K (DK).			
(72) Inventor; and (75) Inventor/Applicant (for US only): POULSEN, Peter [DK/DK]; Danisco a/s, Langebrogade 1, P.O. Box 17, DK-1001 Copenhagen K (DK).			
(74) Agents: MASCHIO, Antonio et al.; D Young & Co., 21 New Fetter Lane, London EC4A 1DA (GB).			

(54) Title: **ANTISENSE INTRON INHIBITION OF STARCH BRANCHING ENZYME EXPRESSION**

(57) Abstract

A method of inhibiting gene expression is described. The method, which affects enzymatic activity in a plant, comprises expressing in a plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in an antisense orientation of a class A SBE; and wherein the nucleotide sequence does not contain a sequence that is antisense to an exon sequence normally associated with the intron.



Best Available Copy

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

ANTISENSE INTRON INHIBITION OF STARCH BRANCHING ENZYME EXPRESSION

The present invention relates to a method of inhibiting gene expression, particularly inhibiting gene expression in a plant. The present invention also relates to a nucleotide
5 sequence useful in the method. In addition, the present invention relates to a promoter that is useful for expressing the nucleotide sequence.

Starch is one of the main storage carbohydrates in plants, especially higher plants. The structure of starch consists of amylose and amylopectin. Amylose consists essentially of straight chains of α -1-4-linked glycosyl residues. Amylopectin comprises chains of
10 α -1-4-linked glycosyl residues with some α -1-6 branches. The branched nature of amylopectin is accomplished by the action of *inter alia* an enzyme commonly known as the starch branching enzyme ("SBE"). SBE catalyses the formation of branch points in the amylopectin molecule by adding α -1,4 glucans through α -1,6-glucosidic branching linkages. The biosynthesis of amylose and amylopectin is schematically shown in Figure
15 1, whereas the α -1-4-links and the α -1-6 links are shown in Figure 2.

In Potato, it is known that two classes of SBE exist. In our copending international patent applications PCT/EP96/03052 and PCT/EP96/03053, class B potato SBE and a gene encoding it are discussed. In international patent application
20 WO96/34968, class A potato SBE and a cDNA encoding it are disclosed.

It is known that starch is an important raw material. Starch is widely used in the food, paper, and chemical industries. However, a large fraction of the starches used in these industrial applications are post-harvest modified by chemical, physical or enzymatic methods in order to obtain starches with certain required functional properties.

Within the past few years it has become desirable to make genetically modified
25 plants which could be capable of producing modified starches which could be the same as the post-harvest modified starches. It is also known that it may be possible to prepare such genetically modified plants by expression of antisense nucleotide coding sequences. In this regard, June Bourque provides a detailed summary of antisense strategies for the genetic manipulations in plants (Bourque 1995 Plant Science 105 pp 125-149). At this
30 stage, reference could be made to Figure 3 which is a schematic diagram of one of the proposed mechanisms of antisense-RNA inhibition.

In particular, WO 92/11375 reports on a method of genetically modifying potato so as to form amylose-type starch. The method involves the use of an anti-sense construct that can apparently inhibit, to a varying extent, the expression of the gene coding for formation of the branching enzyme in potato. The antisense construct of WO 92/11375 consists of a tuber specific promoter, a transcription start sequence and the first exon of the branching enzyme in antisense direction. However, WO 92/11375 does not provide any antisense sequence data. In addition, WO 92/11375 only discloses the use of the potato GBSS promoter.

WO 92/14827 reports on a plasmid that, after insertion into the genome of a plant, can apparently cause changes in the carbohydrate concentration and carbohydrate composition, such as the concentration and composition of amylose and amylopectin, in the regenerated plant. The plasmid contains part of the coding sequence of a branching enzyme in an antisense orientation.

EP-A-0647715 reports on the use of antisense endogenous mRNA coding DNA to alter the characteristics and the metabolic pathways of ornamental plants.

EP-A-0467349 reports on the expression of sequences that are antisense to sequences upstream of a promoter to control gene expression.

EP-A-0458367 and US-A-5107065 report on the expression of a nucleotide sequence to regulate gene expression in a plant. The nucleotide sequence is complementary to a mRNA sequence of a gene and may cover all or a portion of the non-coding region of the gene. In other words, the nucleotide sequences of EP-A-0458367 and US-A-5107065 must at least comprise a sequence that is complementary to a coding region. EP-A-0458367 and US-A-5107065 contain minimal sequence information.

WO96/34968 discusses the use of antisense sequences complementary to sequences which encode class A and class B potato SBE to downregulate SBE expression in potato plants. The sequences used are complementary to SBE coding sequences.

Kuipers *et al* in Mol. Gen. Genet. [1995] 246 745-755 report on the expression of a series of nucleotides that are antisense to part of the genomic intron sequences of potato granule bound starch synthetase. Here the antisense intron sequences are attached to a part of the antisense exon sequences - wherein the intron sequences and the exon

sequences are naturally associated with each other. In addition, the expressed antisense intron sequences are at most 231 bp in length.

Likewise, Kull *et al* in J. Genet. & Breed. [1995] 49 69-76 report on the expression of a series of nucleotides that are antisense to part of the genomic intron sequences of potato granule bound starch synthetase. Likewise, here the antisense intron sequences are attached to a part of the antisense exon sequences - wherein the intron sequences and the exon sequences are naturally associated with each other. In addition, likewise, the expressed antisense intron sequences are at most 231 bp in length.

Shimada *et al* in Theor. Appl. Genet. [1993] 86 665-672 report on the expression of a series of nucleotides that are antisense to part of the genomic intron sequences of rice granule bound starch synthetase. Here the antisense intron sequences are attached to a part of the antisense exon sequences - wherein the intron sequences and the exon sequences are naturally associated with each other. In addition, the expressed antisense intron sequences are less than 350 bp in length.

Reviews on how enzymatic activity can be affected by expression of particular nucleotide sequences may be found in the teachings of Finnegan and McElroy [1994] Biotechnology 12 883-888; and Matzke and Matzke [1995] TIG 11 1-3.

Whilst it is known that enzymatic activity can be affected by expression of particular nucleotide sequences there is still a need for a method that can more reliably and/or more efficiently and/or more specifically affect enzymatic activity.

According to a first aspect of the present invention there is provided a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence partially or completely codes for (is) an intron of the potato class A SBE gene in an antisense orientation optionally together with a nucleotide sequence which codes, partially or completely, for an intron of a class B starch branching enzyme in an antisense or sense orientation; and wherein the nucleotide sequence does not contain a sequence that is antisense to an exon sequence normally associated with the intron.

According to a second aspect of the present invention there is provided a method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an

organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron of the potato class A SBE gene, in an antisense orientation optionally together with a nucleotide sequence which codes, partially or completely, for an intron of a class B starch branching enzyme in an antisense or sense orientation; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

Preferably, the class A SBE gene antisense intron construct is used in combination with a potato class B SBE gene antisense intron construct as defined in PCT/EP96/03052. However, it may also be used independently thereof, to target class A SBE alone, or in combination with other transgenes, to further manipulate starch quality in potato plants.

According to a third aspect of the present invention, therefore, there is provided an antisense sequence comprising the nucleotide sequence shown as any one of SEQ.I.D. No. 15 to SEQ.I.D. No. 27 and the complement of SEQ. ID. No.38, or a variant, derivative or homologue thereof.

According to a fourth aspect of the present invention there is provided a promoter comprising the sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.

According to a fifth aspect of the present invention there is provided a construct capable of comprising or expressing the present invention.

According to a sixth aspect of the present invention there is provided a vector comprising or expressing the present invention.

According to a seventh aspect of the present invention there is provided a cell, tissue or organ comprising or expressing the present invention.

According to an eighth aspect of the present invention there is provided a transgenic starch producing organism comprising or expressing the present invention.

According to a ninth aspect of the present invention there is provided a starch obtained from the present invention.

According to a tenth aspect of the present invention there is provided pSS17 and pSS18.

According to an eleventh aspect of the present invention there is provided a nucleotide sequence that is antisense to any one or more of the intron sequences obtainable from class A SBE, and especially those obtainable from intron 1 of class A SBE as set forth in SEQ. ID. No. 38.

5 A key advantage of the present invention is that it provides a method for preparing modified starches that is not dependent on the need for post-harvest modification of starches. Thus the method of the present invention obviates the need for the use of hazardous chemicals that are normally used in the post-harvest modification of starches.

10 In addition, the present invention provides *inter alia* genetically modified plants which are capable of producing modified and/or novel and/or improved starches whose properties would satisfy various industrial requirements.

Thus, the present invention provides a method of preparing tailor-made starches in plants which could replace the post-harvest modified starches.

15 Also, the present invention provides a method that enables modified starches to be prepared by a method that can have a more beneficial effect on the environment than the known post-harvest modification methods which are dependent on the use of hazardous chemicals and large quantities of energy.

20 An other key advantage of the present invention is that it provides a method that may more reliably and/or more efficiently and/or more specifically affect enzymatic activity when compared to the known methods of affecting enzymatic activity. With regard to this advantage of the present invention it is to be noted that there is some degree of homology between coding regions of SBEs. However, there is little or no homology with the intron sequences of SBEs.

25 Thus, antisense intron expression provides a mechanism to affect selectively the expression of a particular class A SBE. This advantageous aspect could be used, for example, to reduce or eliminate a particular SBE enzyme, especially a class A SBE enzyme, and replace that enzyme with another enzyme which can be another branching enzyme or even a recombinant version of the affected enzyme or even a hybrid enzyme which could for example comprise part of a SBE enzyme from one source and at least
30 part of another SBE enzyme from another source. This particular feature of the present

invention is covered by the combination aspect of the present invention which is discussed in more detail later.

Thus the present invention provides a mechanism for selectively affecting class A SBE activity. This is in contrast to the prior art methods which are dependent on the use of for example antisense exon expression whereby it would not be possible to introduce new SBE activity without affecting that activity as well.

In the context of the present invention, class B SBE is synonymous with SBE I: class A SBE is synonymous with SBE II. Class A SBE is as defined in WO96/34968, incorporated herein by reference. Preferably, the antisense intron construct used comprises intron 1 of class A SBE, which is 2.0 kb in length and is located starting at residue 45 of the coding sequence of class A SBE. The boundaries of the intron may be calculated by searching for consensus intron boundary sequences, and are shown in attached figure 13. Class B SBE is substantially as defined in the sequences given herein and in PCT/EP96/03052.

Preferably with the first aspect of the present invention starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

Preferably with the second aspect of the present invention the nucleotide sequence does not contain a sequence that is antisense to an exon sequence normally associated with the intron.

Preferably with the fourth aspect of the present invention the promoter is in combination with a gene of interest ("GOI").

Preferably the enzymatic activity is reduced or eliminated.

Preferably the nucleotide sequence codes for at least substantially all of at least one intron in an antisense orientation.

Preferably the nucleotide sequence codes, partially or completely, for two or more introns and wherein each intron is in an anti-sense orientation.

Preferably the nucleotide sequence comprises at least 350 nucleotides (e.g. at least 350 bp), more preferably at least 500 nucleotides (e.g. at least 500 bp).

Preferably the nucleotide sequence comprises the complement of the sequence shown in SEQ. ID. No. 38, or a fragment thereof.

Preferably the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ. I.D. No 14 or a variant, derivative or homologue thereof.

Preferably the transgenic starch producing organism is a plant.

A preferred aspect of the present invention therefore relates to a method of
5 affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising
expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence
wherein the nucleotide sequence codes, partially or completely, for an intron in an
antisense orientation; wherein the nucleotide sequence does not contain a sequence that is
antisense to an exon sequence normally associated with the intron; and wherein starch
10 branching enzyme activity is affected and/or the levels of amylopectin are affected and/or
the composition of starch is changed.

A more preferred aspect of the present invention therefore relates to a method of
affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising
expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence
15 wherein the nucleotide sequence codes, partially or completely, for an intron in an
antisense orientation; wherein the nucleotide sequence does not contain a sequence that is
antisense to an exon sequence normally associated with the intron; wherein starch
branching enzyme activity is affected and/or the levels of amylopectin are affected and/or
the composition of starch is changed; and wherein the nucleotide sequence comprises the
20 sequence shown as any one of SEQ.I.D. No. 15 to SEQ.I.D. No. 27 or a variant,
derivative or homologue thereof, including combinations thereof.

The term "nucleotide" in relation to the present invention includes DNA and
RNA. Preferably it means DNA, more preferably DNA prepared by use of recombinant
DNA techniques.

25 The term "intron" is used in its normal sense as meaning a segment of
nucleotides, usually DNA, that is transcribed but does not encode part or all of an
expressed protein or enzyme.

The term "exon" is used in its normal sense as meaning a segment of nucleotides,
usually DNA, encoding part or all of an expressed protein or enzyme.

30 Thus, the term "intron" refers to gene regions that are transcribed into RNA
molecules, but which are spliced out of the RNA before the RNA is translated into a

protein. In contrast, the term "exon" refers to gene regions that are transcribed into RNA and subsequently translated into proteins.

The terms "variant" or "homologue" or "fragment" in relation to the nucleotide sequence of the present invention include any substitution of, variation of, modification
5 of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective nucleotide sequence providing the resultant nucleotide sequence can affect enzyme activity in a plant, or cell or tissue thereof, preferably wherein the resultant nucleotide sequence has at least the same effect as the complement of the sequence shown as SEQ.I.D. No. 38. In particular, the term "homologue" covers homology with respect
10 to similarity of structure and/or similarity of function providing the resultant nucleotide sequence has the ability to affect enzymatic activity in accordance with the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least
15 98% homology. The above terms are also synonymous with allelic variations of the sequences.

Likewise, the terms "variant" or "homologue" or "fragment" in relation to the promoter of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the
20 respective promoter sequence providing the resultant promoter sequence allows expression of a GOI, preferably wherein the resultant promoter sequence has at least the same effect as SEQ.I.D. No. 14. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant promoter sequence has the ability to allow for expression of a GOI, such as a nucleotide
25 sequence according to the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

The term "antisense" means a nucleotide sequence that is complementary to, and can therefore hybridise with, any one or all of the intron sequences of the present invention, including partial sequences thereof.

With the present invention, the antisense intron can be complementary to an entire
5 intron of the gene to be inhibited. However, in some circumstances, partial antisense sequences may be used (i.e. sequences that are not or do not comprise the full complementary sequence) providing the partial sequences affect enzymatic activity. Suitable examples of partial sequences include sequences that are shorter than the full complement of SEQ. ID. No. 38 but which comprise nucleotides that are at least
10 antisense to the sense intron sequences adjacent the respective exon or exons.

With regard to the second aspect of the present invention (i.e. specifically affecting SBE activity), the nucleotide sequences of the present invention may comprise one or more sense or antisense exon sequences of the SBE gene, including complete or partial sequences thereof, providing the nucleotide sequences can affect SBE activity,
15 preferably wherein the nucleotide sequences reduce or eliminate SBE activity. Preferably, the nucleotide sequence of the second aspect of the present invention does not comprise an antisense exon sequence.

The term "vector" includes an expression vector and a transformation vector. The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression. The
20 term "transformation vector" means a construct capable of being transferred from one species to another - such as from an *E. Coli* plasmid to a fungus or a plant cell, or from an *Agrobacterium* to a plant cell.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - in relation to the antisense nucleotide sequence aspect of the
25 present invention includes the nucleotide sequence according to the present invention directly or indirectly attached to a promoter. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the *ShI*-intron or the ADH intron, intermediate the promoter and the nucleotide sequence of the present invention. The same is true for the term "fused" in relation to the present invention
30 which includes direct or indirect attachment. The terms do not cover the natural

combination of the wild type SBE gene when associated with the wild type SBE gene promoter in their natural environment.

The construct may even contain or express a marker which allows for the selection of the genetic construct in, for example, a plant cell into which it has been transferred. Various markers exist which may be used in, for example, plants - such as mannose. Other examples of markers include those that provide for antibiotic resistance - e.g. resistance to G418, hygromycin, bleomycin, kanamycin and gentamycin.

The construct of the present invention preferably comprises a promoter. The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site in the Jacob-Monod theory of gene expression. Examples of suitable promoters are those that can direct efficient expression of the nucleotide sequence of the present invention and/or in a specific type of cell. Some examples of tissue specific promoters are disclosed in WO 92/11375.

The promoter could additionally include conserved regions such as a Pribnow Box or a TATA box. The promoters may even contain other sequences to affect (such as to maintain, enhance, decrease) the levels of expression of the nucleotide sequence of the present invention. Suitable examples of such sequences include the *Sh1*-intron or an ADH intron. Other sequences include inducible elements - such as temperature, chemical, light or stress inducible elements. Also, suitable elements to enhance transcription or translation may be present. An example of the latter element is the TMV 5' leader sequence (see Sleat Gene 217 [1987] 217-225; and Dawson Plant Mol. Biol. 23 [1993] 97).

As mentioned, the construct and/or the vector of the present invention may include a transcriptional initiation region which may provide for regulated or constitutive expression. Any suitable promoter may be used for the transcriptional initiation region, such as a tissue specific promoter. In one aspect, preferably the promoter is the patatin promoter or the E35S promoter. In another aspect, preferably the promoter is the SBE promoter.

If, for example, the organism is a plant then the promoter can be one that affects expression of the nucleotide sequence in any one or more of seed, tuber, stem, sprout, root and leaf tissues, preferably tuber. By way of example, the promoter for the

nucleotide sequence of the present invention can be the α -Amy 1 promoter (otherwise known as the Amy 1 promoter, the Amy 637 promoter or the α -Amy 637 promoter) as described in our co-pending UK patent application No. 9421292.5 filed 21 October 1994. Alternatively, the promoter for the nucleotide sequence of the present invention can be the

5 α -Amy 3 promoter (otherwise known as the Amy 3 promoter, the Amy 351 promoter or the α -Amy 351 promoter) as described in our co-pending UK patent application No. 9421286.7 filed 21 October 1994.

The present invention also encompasses the use of a promoter to express a nucleotide sequence according to the present invention, wherein a part of the promoter is

10 inactivated but wherein the promoter can still function as a promoter. Partial inactivation of a promoter in some instances is advantageous. In particular, with the Amy 351 promoter mentioned earlier it is possible to inactivate a part of it so that the partially inactivated promoter expresses the nucleotide sequence of the present invention in a more specific manner such as in just one specific tissue type or organ. The term "inactivated"

15 means partial inactivation in the sense that the expression pattern of the promoter is modified but wherein the partially inactivated promoter still functions as a promoter. However, as mentioned above, the modified promoter is capable of expressing a gene coding for the enzyme of the present invention in at least one (but not all) specific tissue of the original promoter. Examples of partial inactivation include altering the folding

20 pattern of the promoter sequence, or binding species to parts of the nucleotide sequence, so that a part of the nucleotide sequence is not recognised by, for example, RNA polymerase. Another, and preferable, way of partially inactivating the promoter is to truncate it to form fragments thereof. Another way would be to mutate at least a part of the sequence so that the RNA polymerase can not bind to that part or another part.

25 Another modification is to mutate the binding sites for regulatory proteins for example the CreA protein known from filamentous fungi to exert carbon catabolite repression, and thus abolish the catabolite repression of the native promoter.

The construct and/or the vector of the present invention may include a transcriptional termination region.

30 The nucleotide according to the present invention can be expressed in combination (but not necessarily at the same time) with an additional construct. Thus the present

invention also provides a combination of constructs comprising a first construct comprising the nucleotide sequence according to the present invention operatively linked to a first promoter; and a second construct comprising a GOI operatively linked to a second promoter (which need not be the same as the first promoter). With this aspect of the present invention the combination of constructs may be present in the same vector, plasmid, cells, tissue, organ or organism. This aspect of the present invention also covers methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a plant. With this aspect of the present invention the second construct does not cover the natural combination of the gene coding for an enzyme ordinarily associated with the wild type gene promoter when they are both in their natural environment.

An example of a suitable combination would be a first construct comprising the nucleotide sequence of the present invention and a promoter, such as the promoter of the present invention, and a second construct comprising a promoter, such as the promoter of the present invention, and a GOI wherein the GOI codes for another starch branching enzyme either in sense or antisense orientation.

The above comments relating to the term "construct" for the antisense nucleotide aspect of the present invention are equally applicable to the term "construct" for the promoter aspect of the present invention. In this regard, the term includes the promoter according to the present invention directly or indirectly attached to a GOI.

The term "GOI" with reference to the promoter aspect of the present invention or the combination aspect of the present invention means any gene of interest, which need not necessarily code for a protein or an enzyme - as is explained later. A GOI can be any nucleotide sequence that is either foreign or natural to the organism in question, for example a plant.

Typical examples of a GOI include genes encoding for other proteins or enzymes that modify metabolic and catabolic processes. The GOI may code for an agent for introducing or increasing pathogen resistance.

The GOI may even be an antisense construct for modifying the expression of natural transcripts present in the relevant tissues. An example of such a GOI is the nucleotide sequence according to the present invention.

The GOI may even code for a protein that is non-natural to the host organism - e.g. a plant. The GOI may code for a compound that is of benefit to animals or humans. For example, the GOI could code for a pharmaceutically active protein or enzyme such as any one of the therapeutic compounds insulin, interferon, human serum albumin, human growth factor and blood clotting factors. The GOI may even code for a protein giving additional nutritional value to a food or feed or crop. Typical examples include plant proteins that can inhibit the formation of anti-nutritive factors and plant proteins that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant). The GOI may even code for an enzyme that can be used in food processing such as xylanases and α -galactosidase. The GOI can be a gene encoding for any one of a pest toxin, an antisense transcript such as that for α -amylase, a protease or a glucanase. Alternatively, the GOI can be a nucleotide sequence according to the present invention.

The GOI can be the nucleotide sequence coding for the arabinofuranosidase enzyme which is the subject of our co-pending UK patent application 9505479.7. The GOI can be the nucleotide sequence coding for the glucanase enzyme which is the subject of our co-pending UK patent application 9505475.5. The GOI can be the nucleotide sequence coding for the α -amylase enzyme which is the subject of our co-pending UK patent application 9413439.2. The GOI can be the nucleotide sequence coding for the α -amylase enzyme which is the subject of our co-pending UK patent application 9421290.9. The GOI can be any of the nucleotide sequences coding for the α -glucan lyase enzyme which are described in our co-pending PCT patent application PCT/EP94/03397.

In one aspect the GOI can even be a nucleotide sequence according to the present invention but when operatively linked to a different promoter.

The GOI could include a sequence that codes for one or more of a xylanase, an arabinase, an acetyl esterase, a rhamnogalacturonase, a glucanase, a pectinase, a branching enzyme or another carbohydrate modifying enzyme or proteinase. Alternatively, the GOI may be a sequence that is antisense to any of those sequences.

As mentioned above, the present invention provides a mechanism for selectively affecting a particular enzymatic activity. In an important application of the present invention it is now possible to reduce or eliminate expression of a genomic nucleotide sequence coding for a genomic protein or enzyme by expressing an antisense intron
5 construct for that particular genomic protein or enzyme and (e.g. at the same time) expressing a recombinant version of that enzyme or protein - in other words the GOI is a recombinant nucleotide sequence coding for the genomic enzyme or protein. This application allows expression of desired recombinant enzymes and proteins in the absence of (or reduced levels of) respective genomic enzymes and proteins. Thus the desired
10 recombinant enzymes and proteins can be easily separated and purified from the host organism. This particular aspect of the present invention is very advantageous over the prior art methods which, for example, rely on the use of anti-sense exon expression which methods also affect expression of the recombinant enzyme.

Thus, a further aspect of the present invention relates to a method of expressing a
15 recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence wherein the further nucleotide sequence codes, partially or completely, for an intron in an antisense orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme
20 corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is antisense to an exon sequence normally associated with the intron. Additional aspects cover the combination of those nucleotide sequences including their incorporation in constructs, vectors, cells, tissues and transgenic organisms.

25 Therefore the present invention also relates to a combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in antisense orientation; wherein the intron is an intron that is associated with a genomic gene encoding an enzyme corresponding to the recombinant enzyme; and wherein the second nucleotide sequence
30 does not contain a sequence that is antisense to an exon sequence normally associated with the intron.

The GOI may even code for one or more introns, such as any one or more of the intron sequences presented in the attached sequence listings. For example, the present invention also covers the expression of for example an antisense intron (e.g. the complement of SEQ. ID. No. 38) in combination with for example a sense intron which
5 preferably is not complementary to the antisense intron sequence (e.g. SEQ.I.D.No. 2 or another class A SBE intron).

The terms "cell", "tissue" and "organ" include cell, tissue and organ *per se* and when within an organism.

The term "organism" in relation to the present invention includes any organism
10 that could comprise the nucleotide sequence according to the present invention and/or wherein the nucleotide sequence according to the present invention can be expressed when present in the organism. Preferably the organism is a starch producing organism such as any one of a plant, algae, fungi, yeast and bacteria, as well as cell lines thereof. Preferably the organism is a plant.

15 The term "starch producing organism" includes any organism that can biosynthesise starch. Preferably, the starch producing organism is a plant.

The term "plant" as used herein includes any suitable angiosperm, gymnosperm, monocotyledon and dicotyledon. Typical examples of suitable plants include vegetables such as potatoes; cereals such as wheat, maize, and barley; fruit; trees; flowers; and other
20 plant crops. Preferably, the term means "potato".

The term "transgenic organism" in relation to the present invention includes any organism that comprises the nucleotide sequence according to the present invention and/or products obtained therefrom, and/or wherein the nucleotide sequence according to the present invention can be expressed within the organism. Preferably the nucleotide
25 sequence of the present invention is incorporated in the genome of the organism. Preferably the transgenic organism is a plant, more preferably a potato.

To prepare the host organism one can use prokaryotic or eukaryotic organisms. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see
30 Sambrook *et al* (Sambrook *et al.* in *Molecular Cloning: A Laboratory Manual*, 2nd edition, 1989, Cold Spring Harbor Laboratory Press).

Even though the enzyme according to the present invention and the nucleotide sequence coding for same are not disclosed in EP-B-0470145 and CA-A-2006454, those two documents do provide some useful background commentary on the types of techniques that may be employed to prepare transgenic plants according to the present invention. Some of these background teachings are now included in the following commentary.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a nucleotide sequence or construct according to the present invention and which is capable of introducing the nucleotide sequence or construct into the genome of an organism, such as a plant.

The vector system may comprise one vector, but it can comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An *et al.* (1980), Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An *et al.* (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. Several different Ti and Ri plasmids have been constructed which are suitable for the construction of the plant or plant cell constructs described above. A non-limiting example of such a Ti plasmid is pGV3850.

The nucleotide sequence or construct of the present invention should preferably be inserted into the Ti-plasmid between the terminal sequences of the T-DNA or adjacent a T-DNA sequence so as to avoid disruption of the sequences immediately surrounding the T-DNA borders, as at least one of these regions appears to be essential for insertion of modified T-DNA into the plant genome.

As will be understood from the above explanation, if the organism is a plant the vector system of the present invention is preferably one which contains the sequences necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct.

Furthermore, the vector system is preferably an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof. As these plasmids are well-known and widely employed in the construction of transgenic plants, many vector systems exist which are based on these plasmids or derivatives thereof.

In the construction of a transgenic plant the nucleotide sequence or construct of the present invention may be first constructed in a microorganism in which the vector can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli*, it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the nucleotide sequence or construct of the present invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the present invention, which DNA is subsequently transferred into the plant cell to be modified.

If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblasterdam, 1985, Chapter V; Fraley, *et al.*, Crit. Rev. Plant Sci., 4:1-46; and An *et al.*, EMBO J. (1985) 4:277-284.

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol
5 [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). With this technique, infection of a plant may be performed in or on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the GOI (such as the nucleotide sequence according to the present invention) and, optionally,
10 a promoter, a plant to be infected is wounded, e.g. by cutting the plant with a razor blade or puncturing the plant with a needle or rubbing the plant with an abrasive. The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then grown on a suitable culture medium and allowed to develop into mature plants.

When plant cells are constructed, these cells may be grown and maintained in
15 accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc.

Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue
20 cultures, for example by selecting transformed shoots using an antibiotic and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

Further teachings on plant transformation may be found in EP-A-0449375.

As reported in CA-A-2006454, a large amount of cloning vectors are available
25 which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR 322, pUC series, M13 mp series, pACYC 184 etc. In this way, the nucleotide or construct of the present invention can be introduced into a suitable restriction position in the vector. The contained plasmid is then used for the transformation in *E. coli*. The *E. coli* cells are cultivated in a suitable
30 nutrient medium and then harvested and lysed. The plasmid is then recovered. As a method of analysis there is generally used sequence analysis, restriction analysis,

electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted and connected with the next DNA sequence. Each sequence can be cloned in the same or different plasmid.

After the introduction of the nucleotide sequence or construct according to the present invention in the plants the presence and/or insertion of further DNA sequences may be necessary - such as to create combination systems as outlined above (e.g. an organism comprising a combination of constructs).

The above commentary for the transformation of prokaryotic organisms and plants with the nucleotide sequence of the present invention is equally applicable for the transformation of those organisms with the promoter of the present invention.

In summation, the present invention relates to affecting enzyme activity by expressing antisense intron sequences.

Also, the present invention relates to a promoter useful for the expression of those antisense intron sequences.

The following samples have been deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited (NCIMB) at 23 St Machar Drive, Aberdeen, Scotland, AB2 1RY, United Kingdom, on 13 July 1995:

NCIMB 40753 (which refers to pBEA 8 as described herein);
NCIMB 40751 (which refers to λ -SBE 3.2 as described herein), and
NCIMB 40752 (which refers to λ -SBE 3.4 as described herein).

The following sample has been deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited (NCIMB) at 23 St Machar Drive, Aberdeen, Scotland, AB2 1RY, United Kingdom, on 9 July 1996:

NCIMB 40815 (which refers to pBEA 9 as described herein).

A highly preferred embodiment of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in an antisense orientation; wherein the nucleotide sequence does not contain a sequence that

is antisense to an exon sequence normally associated with the intron; wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed; and wherein the nucleotide sequence is antisense to intron 1 of class A SBE as set forth in SEQ. ID. No. 38, or any other intron of class A SBE, including fragments thereof, and including combinations of class A antisense intron sequences and class B antisense intron sequences. The sequence of introns of class A SBE other than intron 1 may be obtained by sequencing of, for example, potato class A SBE genomic DNA, isolatable by hybridisation screening of a genomic DNA library with class A SBE cDNA obtainable according to WO96/34968 according to methods well known in the art and set forth, for example, in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1989.

The present invention will now be described only by way of example, in which reference is made to the following attached Figures:

Figure 1, which is a schematic representation of the biosynthesis of amylose and amylopectin;

Figure 2, which is a diagrammatic representation of the α -1-4-links and the α -1-6 links of amylopectin;

Figure 3, which is a diagrammatic representation of a possible antisense-RNA inhibition mechanism;

Figure 4, which is a diagrammatic representation of the exon-intron structure of a genomic SBE clone;

Figure 5, which is a plasmid map of pPATA1, which is 3936 bp in size;

Figure 6, which is a plasmid map of pABE6, which is 5106 bp in size;

Figure 7, which is a plasmid map of pVictorIV Man, which is 7080 bp in size;

Figure 8, which is a plasmid map of pBEA8, which is 9.54 kb in size;

Figure 9, which is a plasmid map of pBEA9, which is 9.54 kb in size;

Figure 10, which is a plasmid map of pBEP2, which is 10.32 kb in size;

Figure 11, which is a plasmid map of pVictor5a, which is 9.12 kb in size;

Figure 12, which shows the full genomic nucleotide sequence for SBE including the promoter, exons and introns;

Figure 13, which shows the positioning of intron 1 in the class A and class B SBE genes;

Figure 14, which shows the sequence of intron 1 of the potato class A SBE;

Figure 15, which shows the structure of pSS17; and

5 Figure 16, which shows the structure of pSS18.

Figures 1 and 2 were referred to above in the introductory description concerning starch in general. Figure 3 was referred to above in the introductory description concerning antisense expression.

As mentioned, Figure 4 is a diagrammatic representation of the exon-intron
10 structure of a genomic SBE clone, the sequence of which is shown in Figure 12. This clone, which has about 11.5 k base pairs, comprises 14 exons and 13 introns. The introns are numbered in increasing order from the 5' end to the 3' end and correspond to SEQ.I.D.No.s 1-13, respectively. Their respective antisense intron sequences are shown as SEQ.I.D.No.s 15-27.

15 In more detail, Figures 4 and 12 present information on the 11478 base pairs of a potato SBE gene. The 5' region from nucleotides 1 to 2082 contain the promoter region of the SBE gene. A TATA box candidate at nucleotide 2048 to 2051 is boxed. The homology between a potato SBE cDNA clone (Poulsen & Kreiberg (1993) Plant Physiol 102: 1053-1054) and the exon DNAs begin at 2083 bp and end at 9666 bp.

20 The homology between the cDNA and the exon DNA is indicated by nucleotides in upper case letters, while the translated amino acid sequences are shown in the single letter code below the exon DNA. Intron sequences are indicated by lower case letters.

Figures 5 to 7 are discussed below. As mentioned, Figure 8 is a plasmid map of pBEA8, which is 9.54 k base pairs in size; and Figure 9 is a plasmid map of pBEA9,
25 which is 9.54 k base pairs in size. Each of pBEA 8 and pBEA 9 comprises an antisense sequence to the first intron sequence of the potato SBE gene. This first intron sequence, which has 1177 base pairs, is shown in Figure 4 and lies between the first exon and the second exon.

30 These experiments and aspects of the present invention are now discussed in more detail.

EXPERIMENTAL PROTOCOL**ISOLATION, SUBCLONING IN PLASMIDS, AND SEQUENCING OF GENOMIC CLASS B SBE CLONES**

5 Various clones containing the potato class B SBE gene are isolated from a Desiree potato genomic library (Clontech Laboratories Inc., Palo Alto CA, USA) using radioactively labelled potato SBE cDNA (Poulsen & Kreiberg (1993) Plant Physiol. 102:1053-1054) as probe. The fragments of the isolated λ -phages containing SBE DNA (λ SBE 3.2 - NCIMB 40751 - and λ SBE-3.4 - NCIMB 40752) are identified by Southern
10 analysis and then subcloned into pBluescript II vectors (Clontech Laboratories Inc., Palo Alto CA, USA). λ SBE 3.2 contains a 15 kb potato DNA insert and λ SBE-3.4 contains a 13 kb potato DNA insert. The resultant plasmids are called pGB3, pGB11, pGB15, pGB16 and pGB25 (see discussion below). The respective inserts are then sequenced using the Pharmacia Autoread Sequencing Kit (Pharmacia. Uppsala) and a A.L.F. DNA
15 sequencer (Pharmacia, Uppsala).

In total, a stretch of 11.5 kb of the class B SBE gene is sequenced. The sequence is deduced from the above-mentioned plasmids, wherein: pGB25 contains the sequences from 1 bp to 836 bp, pGB15 contains the sequences from 735 bp to 2580 bp, pGB16 contains the sequences from 2580 bp to 5093 bp, pGB11 contains the sequences from
20 3348 bp to 7975 bp, and pGB3 contains the sequences from 7533 bp to 11468 bp.

In more detail, pGB3 is constructed by insertion of a 4 kb *EcoRI* fragment isolated from λ SBE 3.2 into the *EcoRI* site of pBluescript II SK (+). pGB11 is constructed by insertion of a 4.7 kb *XhoI* fragment isolated from λ SBE 3.4 into the *XhoI* site of pBluescript II SK (+). pGB15 is constructed by insertion of a 1.7 kb *SpeI* fragment
25 isolated from λ SBE 3.4 into the *SpeI* site of pBluescript II SK (+). pGB16 is constructed by insertion of a 2.5 kb *SpeI* fragment isolated from λ SBE 3.4 into the *SpeI* site of pBluescript II SK (+). For the construction of pGB25 a PCR fragment is produced with the primers

5' GGA ATT CCA GTC GCA GTC TAC ATT AC 3'

30

(SEQ. ID. No.30)

and

5' CGG GAT CCA GAG GCA TTA AGA TTT CTG G 3'

(SEQ. ID. No. 31)

and λ SBE 3.4 as a template.

The PCR fragment is digested with *Bam*HI and *Eco*RI, and inserted in pBluescript
5 II SK (+) digested with the same restriction enzymes.

A class A SBE clone is derived similarly.

CONSTRUCTION OF CLASS B SBE ANTISENSE INTRON PLASMIDS pBEA8 and pBEA9

10 The SBE intron 1 is amplified by PCR using the oligonucleotides:

5' CGG GAT CCA AAG AAA TTC TCG AGG TTA CAT GG 3'

(SEQ. ID. No. 32)

and

5' CGG GAT CCG GGG TAA TTT TTA CTA ATT TCA TG 3'

15 (SEQ. ID. No. 33)

and the λ SBE 3.4 phage containing the SBE gene as template.

The PCR product is digested with *Bam*HI and inserted in an antisense orientation
in the *Bam*HI site of plasmid pPATA1 (described in WO 94/24292) between the patatin
promoter and the 35S terminator. This construction, pABE6, is digested with *Kpn*I, and
20 the 2.4 kb "patatin promoter-SBE intron 1- 35S terminator" *Kpn*I fragment is isolated and
inserted in the *Kpn*I site of the plant transformation vector pVictorIV Man. The *Kpn*I
fragment is inserted in two orientations yielding plasmids pBEA8 and pBEA9. pVictorIV
Man is shown in Figure 7 and is formed by insertion of a filled in *Xba*I fragment
containing a E35S promoter-manA-35S terminator cassette isolated from plasmid
25 pVictorIV SGiN Man (WO 94/24292) into the filled in *Xho*I site of pVictor IV. The
pVictor regions of pVictor IV Man contained between the co-ordinates 2.52 bp to 0.32 bp
(see Figure 7).

CONSTRUCTION OF CLASS A SBE ANTISENSE INTRON PLASMIDS pSS17 and pSS18

Construction of plasmid pSS17.

5 The 2122 bp intron 1 sequence of the potato SBEII gene is amplified by PCR from a genomic SBEII subclone using the primers 5' - CGG GAT CCC GTA TGT CTC ACT GTG TTT GTG GC - 3' (SEQ. ID. No. 34) and 5' - CGG GAT CCC CCT ACA TAC ATA TAT CAG ATT AG - 3' (SEQ. ID. No. 35). The PCR product is digested with BamHI and inserted in antisense orientation after a patatin promoter in the
10 BamHI site of a plant transformation vector in which the NPTII gene is used as selectable marker (see figure 15).

Construction of plasmid pSS18.

15 The 2122 bp intron 1 sequence of the potato SBEII gene is amplified by PCR from a genomic SBEII subclone using the primers 5' - CGG GAT CCC GTA TGT CTC ACT GTG TTT GTG GC - 3' (SEQ. ID. No. 34) and 5' - CGG GAT CCC CCT ACA TAC ATA TAT CAG ATT AG - 3' (SEQ. ID. No. 35). The PCR product is digested with BamHI and inserted in antisense orientation after a patatin promoter in the BamHI site of a plant transformation vector in which the *manA* gene is used as
20 selectable marker (see figure 16).

PRODUCTION OF TRANSGENIC POTATO PLANTS

Axenic stock cultures

25 Shoot cultures of *Solanum tuberosum* 'Bintje' and 'Dianella' are maintained on a substrate (LS) of a formula according to Linsmaier, E.U. and Skoog, F. (1965), *Physiol. Plant.* 18: 100-127, in addition containing 2 μ M silver thiosulphate at 25°C and 16 h light/8 h dark.

 The cultures are subcultured after approximately 40 days. Leaves are then cut off the shoots and cut into nodal segments (approximately 0.8 cm) each containing one node.

Inoculation of potato tissues

Shoots from approximately 40 days old shoot cultures (height approximately 5-6 cms) are cut into internodal segments (approximately 0.8 cm). The segments are placed into liquid LS-substrate containing the transformed *Agrobacterium tumefaciens* containing the binary vector of interest. The *Agrobacterium* are grown overnight in YMB-substrate (di-potassium hydrogen phosphate, trihydrate (0.66 g/l); magnesium sulphate, heptahydrate (0.20 g/l); sodium chloride (0.10 g/l); mannitol (10.0 g/l); and yeast extract (0.40 g/l)) containing appropriate antibiotics (corresponding to the resistance gene of the *Agrobacterium* strain) to an optical density at 660 nm (OD-660) of approximately 0.8, centrifuged and resuspended in the LS-substrate to an OD-660 of 0.5.

The segments are left in the suspension of *Agrobacterium* for 30 minutes and then the excess of bacteria are removed by blotting the segments on sterile filter paper.

Co-cultivation

The shoot segments are co-cultured with bacteria for 48 hours directly on LS-substrate containing agar (8.0 g/l), 2,4-dichlorophenoxyacetic acid (2.0 mg/l) and trans-zeatin (0.5 mg/l). The substrate and also the explants are covered with sterile filter papers, and the petri dishes are placed at 25°C and 16 h light/ 8 dark.

"Washing" procedure

After the 48 h on the co-cultivation substrate the segments are transferred to containers containing liquid LS-substrate containing 800 mg/l carbenicillin. The containers are gently shaken and by this procedure the major part of the *Agrobacterium* is either washed off the segments and/or killed.

Selection

After the washing procedure the segments are transferred to plates containing the LS-substrate, agar (8 g/l), trans-zeatin (1-5 mg/l), gibberellic acid (0.1 mg/l), carbenicillin (800 mg/l), and kanamycin sulphate (50-100 mg/l) or phosphinotricin (1-5 mg/l) or mannose (5 g/l) depending on the vector construction used. The segments are sub-cultured to fresh substrate each 3-4 weeks.

In 3 to 4 weeks, shoots develop from the segments and the formation of new shoots continued for 3-4 months.

Rooting of regenerated shoots

5 The regenerated shoots are transferred to rooting substrate composed of LS-substrate, agar (8 g/l) and carbenicillin (800 mg/l).

 The transgenic genotype of the regenerated shoot is verified by testing the rooting ability on the above mentioned substrates containing kanamycin sulphate (200 mg/l), by performing NPTII assays (Radke, S. E. et al, Theor. Appl. Genet. (1988), 75: 685-694)
10 or by performing PCR analysis according to Wang *et al* (1993, NAR 21 pp 4153-4154). Plants which are not positive in any of these assays are discarded or used as controls. Alternatively, the transgenic plants could be verified by performing a GUS assay on the co-introduced β -glucuronidase gene according to Hodal, L. *et al.* (Pl. Sci. (1992), 87: 115-122).

15

Transfer to soil

 The newly rooted plants (height approx. 2-3 cms) are transplanted from rooting substrate to soil and placed in a growth chamber (21°C, 16 hour light 200-400uE/m²/sec). When the plants are well established they are transferred to the greenhouse, where they
20 are grown until tubers had developed and the upper part of the plants are senescing.

Harvesting

 The potatoes are harvested after about 3 months and then analysed.

25 **BRANCHING ENZYME ANALYSIS**

 The class A and class B SBE expression in the transgenic potato lines is measured using the SBE assays described by Blennow and Johansson (Phytochemistry (1991) 30:437-444) and by standard Western procedures using antibodies directed against potato SBE.

30

STARCH ANALYSIS

Starch is isolated from potato tubers and analysed for the amylose:amylopectin ratio (Hovenkamp-Hermelink et al. (1988) Potato Research 31:241-246). In addition, the chain length distribution of amylopectin is determined by analysis of isoamylase digested starch on a Dionex HPAEC.

The number of reducing ends in isoamylase digested starch is determined by the method described by N. Nelson (1944) J. Biol.Chem. 153:375-380.

The results reveal that there is a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch SBE in the transgenic plants.

CONSTRUCTION OF SBE PROMOTER CONSTRUCT

An SBE promoter fragment is amplified from λ -SBE 3.4 using primers:

5' CCA TCG ATA CTT TAA GTG ATT TGA TGG C 3'
(SEQ. ID. No. 36)

and

5' CGG GAT CCT GTT CTG ATT CTT GAT TTC C 3'.
(SEQ. ID. No. 37)

The PCR product is digested with *ClaI* and *BamHI*. The resultant 1.2 kb fragment is then inserted in pVictor5a (see Figure 11) linearised with *ClaI* and *BglII* yielding pBEP2 (see Figure 10).

STARCH BRANCHING ENZYME MEASUREMENTS OF POTATO TUBERS

Potatoes from potato plants transformed with pBEA8, pBEA9, pSS17 or pSS18 are cut in small pieces and homogenised in extraction buffer (50 mM Tris-HCl pH 7.5, Sodium-dithionite (0.1 g/l), and 2 mM DTT) using a Ultra-Turax homogenizer; 1 g of Dowex xl. is added pr. 10 g of tuber. The crude homogenate is filtered through a miracloth filter and centrifuged at 4°C for 10 minutes at 24.700 g. The supernatant is used for starch branching enzyme assays.

The starch branching enzyme assays are carried out at 25°C in a volume of 400 μ l composed of 0.1 M Na citrate buffer pH 7.0, 0.75 mg/ml amylose, 5 mg/ml bovine serum albumin and the potato extract. At 0, 15, 30 and 60 minutes aliquots of 50 μ l are

removed from the reaction into 20 μ l 3 N HCl. 1 ml of iodine solution is added and the decrease in absorbance at 620 nm is measured with an ELISA spectrophotometer.

The starch branching enzyme (SBE) levels are measured in tuber extracts from 34 transgenic Dianella potato plants transformed with plasmid pBEA8, pSS17 and pSS18.

5 The transformed transgenic lines produce tubers which have SBE levels that are 10% to 15% of the appropriate class A or class B SBE levels found in non transformed Dianella plants.

In a further experiment, plasmids pSS17 and pBEA8 are cotransfected into potato plants, as described above. In the cotransfectants, when analysed as set forth above,
10 simultaneous reduction of class A and class B SBE levels are observed.

SUMMATION

The above-mentioned examples relate to the isolation, sequencing and utilisation of antisense intron constructs derived from a gene for potato class A and class B SBE.
15 These SBE intron antisense constructs can be introduced into plants, such as potato plants. After introduction, a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch in plants can be achieved.

Without wishing to be bound by theory it is believed that the expressed anti-sense nucleotide sequence of the present invention binds to sense introns on pre-mRNA and
20 thereby prevents pre-mRNA splicing and/or subsequent translation of mRNA. This binding therefore is believed to reduce the level of plant enzyme activity (in particular class A and class B SBE activity), which in turn for SBE activity is believed to influence the amylose:amylopectin ratio and thus the branching pattern of amylopectin.

Thus, the present invention provides a method wherein it is possible to manipulate
25 the starch composition in plants, or tissues or cells thereof, such as potato tubers, by reducing the level of SBE activity by using an antisense-RNA technique using antisense intron sequences.

The simultaneous reduction or elimination of class A and class B SBE sequences from the doubly transformed potato plants, moreover, offers the possibility to transform
30 such plants with different SBE genes at will, thus allowing the manipulation of branching in starch according to the desired result.

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the present invention.

The following pages present a number of sequence listings which have been consecutively numbered from SEQ.I.D. No. 1 - SEQ.I.D. No. 38. In brief, SEQ.I.D. No. 1 - SEQ.I.D. No. 13 represent sense intron sequences (genomic DNA); SEQ.I.D. No. 14 represents the SBE promoter sequence (genomic sequence); SEQ.I.D. No. 15 - SEQ.I.D. No. 27 represent antisense intron sequences; and SEQ. I.D. No. 28 represents is the sequence complementary to the SBE promoter sequence - i.e. the SBE promoter sequence in antisense orientation. The full genomic nucleotide sequence for class B SBE including the promoter, exons and introns is shown as SEQ. I.D. No. 29 and is explained by way of Figures 4 and 12 which highlight particular gene features. SEQ. ID. No. 30 to 37 show primers used in the methods set forth above. SEQ. ID. No. 38 shows the sequence of intron 1 of class A SBE.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT:

(A) NAME: DANISCO A/S

(B) STREET: LANGEBROGADE 1

10 (C) CITY: COPENHAGEN K

(E) COUNTRY: DENMARK

(F) POSTAL CODE (ZIP): DK-1001

(ii) TITLE OF INVENTION: INHIBITION OF GENE EXPRESSION

15 (iii) NUMBER OF SEQUENCES: 38

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

20 (C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1165 base pairs

(B) TYPE: nucleic acid

30 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

35 (iv) ANTI-SENSE: NO

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTAAATTTTCTAATTTTCAT GTTAATTTCA ATTATTTTTC GCCTTTGCAT TTCATTTTCC 60

45 AATATATCTG GATCATCTCC TTAGTTTTTT ATTTTATTTT TTATAATATC AAATATGGAA 120

GAAAAATGAC ACTTGTAAG CCATATGTAA GATCATGTG ACAAATTGTC AAGGTGGTTG 180

AGTGTATAAA ATTCAAAAAT TGAGAGATGG AGGGGGGGTG GGGGAAGACA ATATTTAGAA 240

50 AGAGTGTCT AGGAGGTTAT GGAGGACACG GATGAGGGGT AGAAGGTTAG TTAGGTATTT 300

GAGTGTGTC TGGCTTATCC TTTCATACTA GTAGTCGTGG AATTATTTGG GTAGTTTCTT 360

55 GTTTTGTTAT TTGATCTTGG TTATCTATT TTCTGTTTCT TGTACTTCGA TTATTGTATT 420

ATATATCTTG TCGTAGTTAT TGTTCTCGG TAAGAATGCT CTAGCATGCT TCCTTTAGTG 480

TTTTATCATG CCTTCTTTAT ATTCGCGTTG CTTTGAAATG CTTTACTTT AGCCGAGGGT 540
 CTATTAGAAA CAATCTCTCT ATCTCGTAAG GTAGGGGTAA AGTCCTCACC AACTCCACT 600
 5 TGTGGGATTA CATGTGTTT GTTGTGTAA ATCAATTATG TATACATAAT AAGTGGATTT 660
 TTTACAACAC AAATACATGG TCAAGGGCAA AGTCTGAAC ACATAAAGGG TTCATTATAT 720
 10 GTCCAGGGAT ATGATAAAAA TTGTTTCTTT GTGAAAGTTA TATAAGATTT GTTATGGCTT 780
 TTGCTGGAAA CATAATAAGT TATAATGCTG AGATAGCTAC TGAAGTTTGT TTTTCTAGC 840
 CTTTAAATG TACCAATAAT AGATTCCGTA TCGAACGAGT ATGTTTGTAT TACCTGGTCA 900
 15 TGATGTTTCT ATTTTTTACA TTTTGTGGT GTTGAAGTGC AATTGAAAAT GTTGTATCCT 960
 ATGAGACGGA TAGTTGAGAA TGTGTTCTTT GTATGGACCT TGAGAAGCTC AAACGCTACT 1020
 20 CCAATAATTT CTATGAATTC AAATTCAGTT TATGGCTACC AGTCAGTCCA GAAATTAGGA 1080
 TATGCTGCAT ATACTTGTTT AATTATACTG TAAAATTTCT TAAGTTCTCA AGATATCCAT 1140
 GTAACCTCGA GAATTTCTTT GACAG 1165
 25

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 317 base pairs
 30 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: DNA (genomic)
 35 (iii) HYPOTHETICAL: NO
 (iv) ANTI-SENSE: NO
 40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

45 GTATGTTTGA TAATTTATAT GGTGTCATGG ATAGTATATA AATAGTTGGA AAATTCTGG 60
 ACTGGTGCTC ATGGCATATT TGATCTGTGC ACCGTGTGGA GATGTCAAAC ATGTGTTACT 120
 TCGTTCCGCC AATTATAAT ACCTTAACTT GGGAAAGACA GCTCTTTACT CCTGTGGGCA 180
 50 TTTGTTATTT GAATTACAAT CTTTATGAGC ATGGTGTGTTT CACATTATCA ACTTCTTTCA 240
 TGTGGTATAT AACAGTTTTT AGCTCCGTTA ATACCTTTCT TCTTTTGTAT ATAACTAAC 300
 55 TGTGGTGCAT TGCTTGC 317

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 504 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTAACAGCCA AAGTTGTGC TTTAGGCAGT TTGACCTTAT TTTGGAAGAT GAATTGTTTA 60
TACCTACTTT GACTTTGCTA GAGAATTTTG CATACCGGGG AGTAAGTAGT GGCTCCATTT 120
AGGTGGCACC TGGCCATTTT TTTGATCTTT TAAAAAGCTG TTTGATTGGG TCTTCAAAAA 180
AGTAGACAAG GTTTTGGAG AAGTGACACA CCCCCGGAGT GTCAGTGGCA AAGCAAAGAT 240
TTTCACTAAG GAGATTCAAA ATATAAAAAA AGTATAGACA TAAAGAAGCT GAGGGGATTC 300
AACATGTACT ATACAAGCAT CAAATATAGT CTTAAAGCAA TTTTGTAGAA ATAAAGAAAG 360
TCTTCTTCT GTTGCTTCAC AATTCCTTC TATTATCATG AGTTACTCTT TCTGTTGAA 420
ATAGCTTCTT TAATATTAAA TTCATGATAC TTTTGTGAG ATTTAGCAGT TTTTCTTGT 480
GTAAACTGCT CTCTTTTTTT GCAG 504

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTAGGTCCTC GTCTACTACA AAATAGTAGT TTCCATCATC ATAACAGATT TTCCTATTAA 60

AGCATGATGT TGCAGCATCA TTGGCTTTCT TACATGTTCT AATTGCTATT AAGGTTATGC 120
TTCTAATTAA CTCATCCACA ATGCAG 146

5 (2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 218 base pairs
(B) TYPE: nucleic acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

25 GTTTTGTTAT TCATACCTTG AAGCTGAATT TTGAACACCA TCATCACAGG CATTCGATT 60
CATGTTCTTA CTAGTCTTGT TATGTAAGAC ATTTTGAAAT GCAAAAGTTA AAATAATTGT 120
GTCTTTACTA ATTTGGACTT GATCCCATAC TCTTCCCTT AACAAAATGA GTCAATTCTA 180
30 TAAGTGCTTG AGAACTTACT ACTTCAGCAA TTAAACAG 218

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 198 base pairs
35 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

50 GTATTTTAAA TTTATTCTA CAACTAAATA ATTCTCAGAA CAATTGTTAG ATAGAATCCA 60
AATATATACG TCCTGAAAGT ATAAAAGTAC TTATTTTCGC CATGGGCCTT CAGAATATTG 120
55 GTAGCCGCTG AATATCATGA TAAGTTATTT ATCCAGTGAC ATTTTATGT TCACTCCTAT 180
TATGTCTGCT GGATACAG 198

(2) INFORMATION FOR SEQ ID NO: 7:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- 15 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

20 GTTTGTCGTG TTCTATTGCA TTTTAAGGTT CATATAGGTT AGCCACGGAA AATCTCACTC 60
TTTGTGAGGT AACCAGGGTT CTGATGGATT ATTCAATTTT CTCGTTTATC ATTTGTTTAT 120
25 TCTTTTCATG CATTGTGTTT CTTTTTCAAT ATCCCTCTTA TTTGGAGGTA ATTTTCTCA 180
TCTATTCACT TTTAGCTTCT AACCACAG 208

(2) INFORMATION FOR SEQ ID NO: 8:

- 30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 293 base pairs
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- 40 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

45 GTATGTCCTTA CATCTTTAGA TATTTTGTGA TAATTACAAT TAGTTTGGCT TACTTGAACA 60
50 AGATTCATTC CTCAAAATGA CCTGAACTGT TGAACATCAA AGGGGTGAA ACATAGAGGA 120
AAACAACATG ATGAATGTTT CCATTGTCTA GGGATTCTTA TTATGTTGCT GAGAACAAAT 180
GTCATCTTAA AAAAAACATT GTTTACTTTT TTGTAGTATA GAAGATTACT GTATAGAGTT 240
55 TGCAAGTGTG TCTGTTTGG AGTAATTGTG AAATGTTTGA TGAACCTGTA CAG 293

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 376 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

20 GTTCAAGTAT TTTGAATCGC AGCTTGTTAA ATAATCTAGT AATTTT TAGA TTGCTTACTT 60
GGAAGTCTAC TTGGTTCTGG GGATGATAGC TCATTTCA TC TTGTTCTACT TATTTTCCAA 120
25 CCGAATTCTCT GATTTTTGTT TCGAGATCCA AGTATTAGAT TCATTTACAC TTATTACCGC 180
CTCATTCTCTA CCACTAAGGC CTTGATGAGC AGCTTAAGTT GATTCTTTGA AGCTATAGTT 240
TCAGGCTACC AATCCACAGC CTGCTATATT TGTGGATAC TTACCTTTTC TTTACAATGA 300
30 AGTGATACTA ATTGAAATGG TCTAAATCTG ATATCTATAT TTCTCCGTCT TTCCTCCCCC 360
TCATGATGAA ATGCAG 376

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTAAATCAT CTAAAGTTGA AAGTGTGGG TTTATGAAGT GCTTTAATTC TATCCAAGGA 60
55 CAAGTAGAAA CCTTTTACC TTCCATTCT TGATGATGGA TTTCATATTA TTTAATCCAA 120
TAGCTGGTCA AATTCGGTAA TAGCTGTACT GATTAGTTAC TTCCTTTGC AG 172

(2) INFORMATION FOR SEQ ID NO: 11:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

20 GTATATATGT TTTACTTATC CATGAAATTA TTGCTCTGCT TGTTTTAAAT GTACTGAACA 60
AGTTTTATGG AGAAGTAACT GAAACAAATC ATTTTCACAT TGTCTAATTT AACTCTTTTT 120
25 TCTGATCCTC GCATGACGAA AACAG 145

(2) INFORMATION FOR SEQ ID NO: 12:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

40 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

45 GTAAGGATTT GCTTGAATAA CTTTGTATAA TAAGATAACA GATGTAGGGT ACAGTTCTCT 60
CACCAAAAAG AACTGTAATT GTCTCATCCA TCTTTAGTTG TATAAGATAT CCGACTGTCT 120
50 GAGTTCGGAA GTGTTTGAGC CTCCTGCCCT CCCCCTGCGT TGTTTAGCTA ATTCAAAAAG 180
GAGAAAAC TG TTTATTGATG ATCTTTGTCT TCATGCTGAC ATACAATCTG TTCTCATGAC 240
AG 242

55

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 797 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
5 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO
10

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
15

GTACAGTTCT TGCCGTGTGA CCTCCCTTTT TATTGTGGTT TTGTTTCATAG TTATTTGAAT	60
20 GCGATAGAAG TTAAC TATTG ATTACCGCCA CAATCGCCAG TTAAGTCCTC TGAAC TACTA	120
ATTTGAAAGG TAGGAATAGC CGTAATAAGG TCTACTTTTG GCATCTTACT GTTACAAAAC	180
25 AAAAGGATGC CAAAAAATT CTTCTCTATC CTC TTTTCC CTAACCAGT GCATGTAGCT	240
TGCACCTGCA TAACTTAGG TAAATGATCA AAAATGAAGT TGATGGGAAC TTAAAACCGC	300
CCTGAAGTAA AGCTAGGAAT AGTCATATAA TGTCCACCTT TGGTGTCTGC GCTAACATCA	360
30 ACAACAACAT ACCTCGTGTA GTCCACAAA GTGGTTTCAG GGGGAGGGTA GAGTGTATGC	420
AAAAC TACT CCTATCTCAG AGGTAGAGAG GATTTTTCAT ATAGACCCTT GGCTCAAGAA	480
35 AAAAAGTCCA AAAAGAAGTA ACAGAAGTGA AAGCAACATG TG TAGCTAAA GCGACCCAAC	540
TTGTTTGGGA CTGAAGTAGT TGTTGTTGTT GAAACAGTGC ATGTAGATGA ACACATGTCA	600
GAAATGGAC AACACAGTTA TTTTGTGCAA GTCAAAAAA TGTACTACTA TTTCTTTGTG	660
40 CAGCTTTATG TATAGAAAAG TTAAATAACT AATGAATTTT GCTAGCAGAA AAATAGCTTG	720
GAGAGAAATT TTTTATATTG AACTAAGCTA ACTATATTCA TCTTTCTTTT TGCTTCTTCT	780
45 TCTCCTTGTT TGTGAAG	797

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2169 base pairs
50 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
55

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

	ATCATGGCCA ATTACTGGTT CAAATGCATT ACTTCCTTTC AGATTCTTTC GAGTTCTCAT	60
10	GACCGGTCCT ACTACAGACG ATACTAACCC GTGGAACGTG TGCATCTGCT TCTTAGAACT	120
	CTATGGCTAT TTTCGTTAGC TTGGCGTCGG TTTGAACATA GTTTTTGTTT TCAAACCTCTT	180
	CATTTACAGT CAAAATGTTG TATGGTTTTT GTTTTCCTCA ATGATGTTTA CAGTGTTGTG	240
15	TTGTCATCTG TACTTTTGCC TATTACTTGT TTTGAGTTAC ATGTTAAAAA AGTGTTTATT	300
	TTGCCATATT TTGTTCTCTT ATTATTATTA TCATACATAC ATTATTACAA GGAAAAGACA	360
20	AGTACACAGA TCTTAACGTT TATGTTCAAT CAACTTTTGG AGGCATTGAC AGGTACCACA	420
	AATTTTGAGT TTATGATTAA GTTCAATCTT AGAATATGAA TTTAACATCT ATTATAGATG	480
	CATAAAAATA GCTAATGATA GAACATTGAC ATTTGGCAGA GCTTAGGGTA TGGTATATCC	540
25	AACGTTAATT TAGTAATTTT TGTTACGTAC GTATATGAAA TATTGAATTA ATCACATGAA	600
	CGGTGGATAT TATATTATGA GTTGGCATCA GCAAATCAT TGGTGTAGTT GACTGTAGTT	660
30	GCAGATTTAA TAATAAAATG GTAATTAACG GTCGATATTA AAATAACTCT CATTTCAAGT	720
	GGGATTAGAA CTAGTTATTA AAAAAATGTA TACTTTAAGT GATTTGATGG CATATAATTT	780
	AAAGTTTTTC ATTTTCATGCT AAAATTGTTA ATTATTGTAA TGTAGACTGC GACTGGAATT	840
35	ATTATAGTGT AAATTTATGC ATTCAGTGTA AAATTAAAGT ATTGAACTTG TCTGTTTTAG	900
	AAAATACTTT ATACTTTAAT ATAGGATTTT GTCATGCGAA TTAAATTAA TCGATATTGA	960
40	ACACGGAATA CCAAATTA TAAGGATACA CATGGCCTTC ATATGAACCG TGAACCTTTG	1020
	ATAACGTGGA AGTTCAAAGA AGGTAAAGTT TAAGAATAAA CTGACAAATT AATTTCTTTT	1080
	ATTTGGCCCA CTAATAAATT TGCTTTACTT TCTAACATGT CAAGTTGTGC CCTCTTAGTT	1140
45	GAATGATATT CATTTTTCAT CCCATAAGTT CAATTTGATT GTCATACCAC CCATGATGTT	1200
	CTGAAAAATG CTTGGCCATT CACAAAGTTT ATCTTAGTTC CTATGAACTT TATAAGAAGC	1260
50	TTTAATTTGA CATGTTATTT ATATTAGATG ATATAATCCA TGACCCAATA GACAAGTGTA	1320
	TTAATATTGT AACTTTGTAA TTGAGTGTGT CTACATCTTA TTCAATCATT TAAGGTCATT	1380
	AAAATAAATT ATTTTTTGAC ATTCTAAAAC TTAAAGCAGA ATAAATAGTT TATCAATTAT	1440
55	TAAAAACAAA AAACGACTTA TTTATAAATC AACAAACAAT TTTAGATTGC TCCAACATAT	1500

TTTTCCAAAT TAAATGCAGA AAATGCATAA TTTTATACTT GATCTTTATA GCTTATTTTT 1560
TTTAGCCTAA CCAACGAATA TTTGTAAACT CACAACTTGA TTAAAAGGGA TTTACAACAA 1620
5 GATATATATA AGTAGTGACA AATCTTGATT TTAAATATTT TAATTTGGAG GTCAAAATTT 1680
TACCATAATC ATTTGTATTT ATAATTAAAT TTAAATATC TTATTTATAC ATATCTAGTA 1740
AACTTTTAA TATACGTATA TACAAAATAT AAAATTATTG GCGTTCATAT TAGGTCAATA 1800
10 AATCCTTAAC TATATCTGCC TTACCACTAG GAGAAAGTAA AAAACTCTTT ACCAAAAATA 1860
CATGTATTAT GTATACAAAA AGTCGATTAG ATTACCTAAA TAGAAATTGT ATAACGAGTA 1920
15 AGTAAGTAGA AATATAAAAA AACTACAATA CTAAAAAAA TATGTTTTAC TTCAATTTTCG 1980
AAACTAATGG GGTCTGAGTG AAATATTCAG AAAGGGGAGG ACTAACAAAA GGGTCATAAT 2040
GTTTTTTTAT AAAAGCCAC TAAAATGAGG AAATCAAGAA TCAGAACATA CAAGAAGGCA 2100
20 GCAGCTGAAG CAAAGTACCA TAATTTAATC AATGGAAATT AATTTCAAAG TTTTATCAAA 2160
ACCCATTCG 2169

25 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1165 base pairs
(B) TYPE: nucleic acid
30 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CTGTCAAAGA AATTCTCGAG GTTACATGGA TATCTTGAGA ACTTAAGAAA TTTTACAGTA 60
45 TAATTGAACA AGTATATGCA GCATATCCTA ATTTCTGGAC TGACTGGTAG CCATAAACTG 120
AATTTGAATT CATAGAAATT ATTGGAGTAG CGTTTGAGCT TCTCAAGGTC CATACAAAGA 180
50 ACACATTCTC AACTATCCGT CTCATAGGAT ACAACATTTT CAATTGCAGT TCAACACCAA 240
AAAAATGTAA AAAATAGAAA CATCATGACC AGGTAATCAA AACATACTCG TTCGATACGG 300
AATCTATTAT TGGTACATTT AAAAGGCTAG AAAAAACAAA CTTCAAGTAGC TATCTCAGCA 360
55 TTATAACTTA TTATGTTTCC AGCAAAAGCC ATAACAAATC TTATATAACT TTCACAAAGA 420

AACAATTTTT ATCATATCCC TGGACATATA ATGAACCCCTT TATGTGTTCA GAACTTTGCC 480
CTTGACCATG TATTTGTGTT GTAAAAATC CACTTATTAT GTATACATAA TTGATTTACA 540
5 ACAACAAACA CAATGTAATC CCACAAGTGG AGTGTGGTGA GGACTTTACC CCTACCTTAC 600
GAGATAGAGA GATTGTTTCT AATAGACCCT CGGCTAAAGT AAAAGCATTT CAAAGCAACG 660
CGAATATAAA GAAGGCATGA TAAACACTA AAGGAAGCAT GCTAGAGCAT TCTTACCGAG 720
10 GAACAATAAC TACGACAAGA TATATAATAC AATAATCGAA GTACAAGAAA CAGAAAATAG 780
AATAACAAAG ATCAAATAAC AAAACAAGAA ACTACCCAAA TAATTCCACG ACTACTAGTA 840
15 TGAAAGGATA AGCCAGACAA CACTCAAATA CCTAACTAAC CTTCTACCCC TCATCCGTGT 900
CCTCCATAAC CTCCTAGAAC ACTCTTTCTA AATATTGTCT TCCCCACCC CCCCTCCATC 960
TCTCAATTTT TGAATTTTAT AACTCAACC ACCTTGCAAA TTTGTCACAT GATACTTACA 1020
20 TATGGCTCTA CAAGTGTCAT TTTTCTTCCA TATTTGATAT TATAAAAAAT AAAATAAAAA 1080
ACTAAGGAGA TGATCCAGAT ATATTGGAAA ATGAAATGCA AAGGCTAAAA ATAATTGAAA 1140
25 TTAACATGAA ATTAGTAAAA ATTAC 1165

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
30 (A) LENGTH: 317 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

40 (iv) ANTI-SENSE: YES

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GCAAGCAATG CACCACAGTT AGTTTATATC AAAAAGAAGA AAGGTATTAA CGGAGCTAAA 60
AACTGTTATA TACCACATGA AAGAAGTTGA TAATGTGAAA ACACCATGCT CATAAAGATT 120
50 GTAATTCAAA TAACAAATGC CCACAGGAGT AAAGAGCTGT CTTTCCCAAG TTAAGGTATT 180
ATAAATTGGC GGAACGAAGT AACACATGTT TGACATCTCC ACACGGTGCA CAGATCAAAT 240
ATGCCATGAG CACCAGTCCA GAAGTTTTC AACTATTTAT ATACTATCCA TGCAACCATA 300
55 TAAATTATCA AACATAC 317

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 504 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CTGCAAAAAA AGAGAGCAGT TTACACAAGA AAAAAGTCTT AAATCTCAAC AAAAGTATCA 60
TGAATTTAAT ATTAAGGAAG CTATTTTCGAA CAGAAAGAGT AACTCATGAT AATAGAAGGA 120
AATTGTGAAG CAACAGAAGG AAGACTTTCT TTATTTCTAC AAAATTGCTT TAAGACTATA 180
TTTGATGCTT GTATAGTACA TGTGAATCC CCTCAGCTTC TTTATGTCTA TACTTTTTTT 240
ATATTTTGAA TCTCCTTAGT GAAAATCTTT GCTTTGCCAC TGACACTCCG GGGGTGTGTC 300
ACTTCTCCAA AAACCTTGTC TACTTTTTTG AAGACCCAAT CAAACAGCTT TTTAAAAGAT 360
CAAAAAAATG GCCAGGTGCC ACCTAAATGG AGCCACTACT TACTCCCCGG TATGCAAAAT 420
TCTCTAGCAA AGTCAAAGTA GGTATAACA ATTCATCTTC CAAAATAAGG TCAAAGTACC 480
TAAAGCACAA CTTTGGCTG TTAC 504

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 146 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CTGCATTGTG GATGAGTTAA TTAGAAGCAT AACCTTAATA GCAATTAGAA CATGTAAGAA 60

AGCCAATGAT GCTGCAACAT CATGCTTTAA TAGGAAAATC TGTATGATG ATGGAAACTA 120
CTATTTTGTA GTAGACGAGG ACCTAC 146

5 (2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 218 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
15 (iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: YES

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

25 CTGTTTAATT GCTGAAGTAG TAAGTTCTCA AGCACTTATA GAATTGACTC ATTTTGTTAA 60
GGGAAAGAGT ATGGGATCAA GTCCAAATTA GTAAAGACAC AATTATTTTA ACTTTTGCAT 120
TTCAAAATGT CTTACATAAC AAGACTAGTA AGAACATGAA TCGAAATGCC TGTGATGATG 180
30 GTGTTCAAAA TTCAGCTTCA AGGTATGAAT AACAAAAC 218

(2) INFORMATION FOR SEQ ID NO: 20:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
45 (iv) ANTI-SENSE: YES

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CTGTATCCAG CAGACATAAT AGGAGTGAAC ATAAAAATGT CACTGGATAA ATAACCTATC 60
ATGATATTCA GCGGCTACCA ATATTCTGAA GGCCCATGGC GAAAATAAGT ACTTTTATAC 120
55 TTTCAGGACG TATATATTTG GATTCTATCT AACAAATTGTT CTGAGAATTA TTTAGTTGTA 180

GAAATAAAATT TAAAATAC

198

(2) INFORMATION FOR SEQ ID NO: 21:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: YES

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTGTGGTTAG AAGCTAAAAG TGAATAGATG AGAAAAATTA CCTCCAAATA AGAGGGATAT 60

TGAAAAAGAA ACACAATGCA TGAAAAGAAT AAACAAATGA TAAACGAGAA AATTGAATAA 120

25 TCCATCAGAA CCCTGGTTAC CTCACAAAGA GTGAGATTTT CCGTGGCTAA CCTATATGAA 180

CCTTAAAATG CAATAGAAAC AGACAAAC 208

30 (2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 293 base pairs
35 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

40 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CTGTACAAGT TCATCAAACA TTTCACAATT ACTCCAAAAC AGACACACTT GCAAACTCTA 60

50 TACAGTAATC TTCTATACTA CAAAAAAGTA AACAATGTTT TTTTAAAGAT GACATTTGTT 120

CTCAGCAACA TAATAGAAAT CCCTAGACAA TGGAAACATT CATCATGTTG TTTTCCTCTA 180

55 TGTTTCAACC CCTTTGATGT TCAACAGTTC AGGTCATTTT GAGGAATGAA TCTTGTTCAA 240

GTAAGCCAAA CTAATTGTAA TTATCACAAA ATATCTAAAG ATGTAAGACA TAC 293

(2) INFORMATION FOR SEQ ID NO: 23:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 376 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

20 CTGCATTTC A TCATGAGGGG GAGGAAAGAC GGAGAAATAT AGATATCAGA TTTAGACCAT 60
TTCAATTAGT ATCACTTCAT TGTAAGAAA AGGTAAGTAT CCAACAAATA TAGCAGGCTG 120
25 TGGATTGGTA GCCTGAAACT ATAGCTTCAA AGAATCAACT TAAGCTGCTC ATCAAGGCCT 180
TAGTGGTAGA AATGAGGCGG TAATAAGTGT AAATGAATCT AATACTTGGA TCTCGAAACA 240
AAAATCAGAA ATTCCGTTGG AAAATAAGTA GAACAAGATG AAATGAGCTA TCATCCCCAG 300
30 AACCAAGTAG ACTTCCAAGT AAGCAATCTA AAAATTACTA GATTATTTAA CAAGCTGCGA 360
TTCAAAATAC TTGAAC 376

35 (2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 base pairs
(B) TYPE: nucleic acid
40 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

45 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

55 CTGCAAAGTG AAGTAACTAA TCAGTACAGC TATTACCGAA TTTGACCAGC TATTGGATTA 60
AATAATATGA AATCCATCAT CAAGAAATGG AAGGTAAAAA GGTTCCTACT TGTCCTTGGA 120

TAGAATTAAA GCACTTCATA AACCCAACAC TTTCAACTTT AGATGATTTT AC

172

(2) INFORMATION FOR SEQ ID NO: 25:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 145 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

15

(iv) ANTI-SENSE: YES

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CTGTTTTCGT CATGCGAGGA TCAGAAAAAA GAGTTAAATT AGACAATGTG AAAATGATTT

60

GTTTCAGTTA CTTCTCCATA AAACCTGTTC AGTACATTAA AAACAAGCAG AGCAATAATT

120

25

TCATGGATAA GTAAAACATA TATAC

145

(2) INFORMATION FOR SEQ ID NO: 26:

- 30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 242 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

40

(iv) ANTI-SENSE: YES

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CTGTCATGAG AACAGATTGT ATGTCAGCAT GAAGACAAAG ATCATCAATA AACAGTTTTC

60

TCCTTTTGA ATTAGCTAAA CAACGCAGGG GGAGGGCAGG AGGCTCAAAC ACTTCCGAAC

120

50

TCAGACAGTC GGATATCTTA TACAACTAAA GATGGATGAG ACAATTACAG TTCTTTTGG

180

TGAGAGAACT GTACCCTACA TCTGTTATCT TATTATCAA AGTTATTCAA GCAAATCCTT

240

55

AC

242

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 797 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CTTCACAAAC AAGGAGAAGA AGAAGCAAAA AGAAAGATGA ATATAGTTAG CTTAGTTCAA	60
TATAAAAAAT TTCTCTCCAA GCTATTTTTT TGCTAGCAAA ATTCATTAGT TATTAACTT	120
TTCTATACAT AAAGCTGCAC AAAGAAATAG TAGTACATTT TTTTGA CTG CACAAAATAA	180
CTGTGTTGTC CATTTTCTGA CATGTGTTCA TCTACATGCA CTGTTTCAAC AACAACT	240
ACTTCAGTCC CAAACAAGTT GGGTCGCTTT AGCTACACAT GTTGCTTTCA CTTCTGTTAC	300
TTCTTTTTGG ACTTTTTTTC TTGAGCCAAG GGTCTATTGA AAAAATCCTC TCTACCTCTG	360
AGATAGGAGT AAGTTTGTGA TACACTCTAC CCTCCCCTG AAACCACTTT GTGGGACTAC	420
ACGAGGTATG TTGTTGTTGA TGTTAGCGCA GACACCAAAG GTGGACATTA TATGACTATT	480
CCTAGCTTTA CTTCAGGGCG GTTTTAAGTT CCCATCAACT TCATTTTGA TCATTTACCT	540
AAGTTTATGC AGGTGCAAGC TACATGCACT GGTTTAGGGA AAAAGAGGAT AGAGAAGAAT	600
TTTTTTGGCA TCCTTTTGTT TTGTAACAGT AAGATGCCAA AAGTAGACCT TATTACGGCT	660
ATTCCTACCT TTCAAATTAG TAGTTCAGAG GACTTAACTG GCGATTGTGG CGGTAATCAA	720
TAGTAACTT CTATCGCATT CAAATAACTA TGAACAAAAC CACAATAAAA AGGGAGGTCA	780
CACGGCAAGA ACTGTAC	797

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2169 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CGAATGGGTT TTGATAAAC TTTGAAATTA ATTTCCATTG ATTAAATTAT GGTACTTTGC	60
10 TTCAGCTGCT GCCTTCTTGT ATGTTCTGAT TCTTGATTTC CTCATTTTAG TGGCTTTTTA	120
TAAAAAACA TTATGACCCT TTTGTTAGTC CTCCTTTTC TGAATATTTC ACTCAGACCC	180
15 CATTAGTTTC GAAATTGAAG TAAAACATAT TTTTTTAGT ATTGTAGTTT TTTTATATTT	240
CTACTTACTT ACTCGTTATA CAATTTCTAT TTAGGTAATC TAATCGACTT TTTGTATACA	300
TAATACATGT ATTTTTGGTA AAGAGTTTTT TACTTTCTCC TAGTGGTAAG GCAGATATAG	360
20 TTAAGGATTT ATTGACCTAA TATGAACGCC AATAATTTTA TATTTGTAT ATACGTATAT	420
TTAAAGTTT ACTAGATATG TATAAATAAG ATATTTAAAA TTTAATTATA AATACAAATG	480
25 ATTATGGTAA AATTTTGACC TCCAAATTAA AATATTTAAA ATCAAGATTT GTCACTACTT	540
ATATATATCT TGTTGTAAAT CCTTTTAAAT CAAGTTGTGA GTTTACAAAT ATTCGTTGGT	600
TAGGCTAAAA AAAATAAGCT ATAAAGATCA AGTATAAAAT TATGCATTTT CTGCATTTAA	660
30 TTTGGAAAAA TATGTTGGAG CAATCTAAAA TTGTTTGTG ATTTATAAAT AAGTCGTTTT	720
TTGTTTTTAA TAATTGATAA ACTATTTATT CTGCTTAAAG TTTTAGAATG TCAAAAAATA	780
35 ATTTATTTTA ATGACCTTAA ATGATTGAAT AAGATGTAGA CACACTCAAT TACAAAGTTA	840
CAATATTAAT ACACCTGTCT ATTGGGTCAT GGATTATATC ATCTAATATA AATAACATGT	900
CAAATTAAG CTTCCTTATA AGTTCATAGG AACTAAGATA AACTTTGTGA ATGGCCAAGC	960
40 ATTTTTCAGA ACATCATGGG TGGTATGACA ATCAAATTGA ACTTATGGGA TGAAAAATGA	1020
ATATCATCA ACTAAGAGGG CACAACCTGA CATGTTAGAA AGTAAAGCAA ATTTAGTAGT	1080
45 GGGCCAAATA AAAGAAATTA ATTTGTCAGT TTATCTTAA ACTTTACCTT CTTTGAACCT	1140
CCACGTTATC AAAGGTTTAC GGTTCATATG AAGGCCATGT GTATCCTTTT TAATTTTGGT	1200
ATTCCGTGTT CAATATCGAT TAATTTAAAT TCGCATGACA AAATCCTATA TTAAAGTATA	1260
50 AAGTATTTTC TAAAACAGAC AAGTTCAATA CTTTAATTTT AACTGAATG CATAAATTTA	1320
CACTATAATA ATTCCAGTCG CAGTCTACAT TACAATAATT AACAATTTTA GCATGAAATG	1380
55 AAAAACTTTA AATTATATGC CATCAAATCA CTTAAAGTAT ACATTTTTTT AATAACTAGT	1440
TCTAATCCCA CTGAAATGA GAGTTATTTT AATATCGACC GTTAATTACC ATTTTATTAT	1500

TAAATCTGCA ACTACAGTCA ACTACACCAA TGATTTTGCT GATGCCAACT CATAATATAA 1560
TATCCACCGT TCATGTGATT AATTCAATAT TTCATATACG TACGTAACAA AAATTACTAA 1620
5 ATTAACGTTG GATATACCAT ACCCTAAGCT CTGCCAAATG TCAATGTTCT ATCATTAGCT 1680
ATTTTATGC ATCTATAATA GATGTTAAAT TCATATTCTA AGATTGAACT TAATCATAAA 1740
10 CTCAAAATTT GTGGTACCTG TCAATGCCTC CAAAAGTTGA TTGAACATAA ACGTTAAGAT 1800
CTGTGTACTT GTCTTTTCCT TGTAATAATG TATGTATGAT AATAATAATA AGAGAACAAA 1860
ATATGGCAA AATAACACTT TTTTAACATG TAACTCAAAA CAAGTAATAG GCAAAAGTAC 1920
15 AGATGACAAC ACAACACTGT AAACATCATT GAGGAAAACA AAAACCATAC AACATTTTGA 1980
CTGTAAATGA AGAGTTTGAA AACAAAAACT ATGTTCAAAC CGACGCCAAG CTAACGAAAA 2040
20 TAGCCATAGA GTTCTAAGAA GCAGATGCAA CAGTTCCACG GGTTAGTATC GTCTGTAGTA 2100
GGACCGGTCA TGAGAACTCG AAAGAATCTG AAAGGAAGTA ATGCATTTGA ACCAGTAATT 2160
GGCCATGAT 2169

25 (2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
30 (A) LENGTH: 11469 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

35 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

45 ATCATGGCCA ATTACTGGTT CAAATGCATT ACTTCCTTTC AGATTCTTTC GAGTTCTCAT 60
GACCGGTCTT ACTACAGACG ATACTAACCC GTGGAACGTG TGCATCTGCT TCTTAGAACT 120
CTATGGCTAT TTTTCGTTAGC TTGGCGTCGG TTTGAACATA GTTTTGTGTT TCAAACCTCTT 180
50 CATTACAGT CAAAATGTTG TATGGTTTTT GTTTTCCTCA ATGATGTITA CAGTGTGTG 240
TTGTCATCTG TACTTTTGCC TATTACTTGT TTTGAGTTAC ATGTTAAAAA AGTGTTTATT 300
55 TTGCCATATT TTGTTCTCTT ATTATTATTA TCATACATAC ATTATTACAA GGAAAAGACA 360
AGTACACAGA TCTTAACGTT TATGTTCAAT CAACTTTTGG AGGCATTGAC AGGTACCACA 420

	AATTTTGAGT TTATGATTAA GTTCAATCTT AGAATATGAA TTTAACATCT ATTATAGATG	480
	CATAAAAATA GCTAATGATA GAACATTGAC ATTTGGCAGA GCTTAGGGTA TGGTATATCC	540
5	AACGTTAATT TAGTAATTTT TGTTACGTAC GTATATGAAA TATTGAATTA ATCACATGAA	600
	CGGTGGATAT TATATTATGA GTTGGCATCA GCAAAATCAT TGGTGTAGTT GACTGTAGTT	660
10	GCAGATTAA TAATAAAATG GTAATTAACG GTCGATATTA AAATAACTCT CATTTCAAGT	720
	GGGATTAGAA CTAGTTATTA AAAAAATGTA TACTTTAAGT GATTTGATGG CATATAATTT	780
	AAAGTTTTTC ATTTTCATGCT AAAATTGTTA ATTATTGTAA TGTAGACTGC GACTGGAATT	840
15	ATTATAGTGT AAATTTATGC ATTCAGTGTA AAATTAAAGT ATTGAAGTTG TCTGTTTTAG	900
	AAAATACTTT ATACTTTAAT ATAGGATTTT GTCATGCGAA TTAAATTA TCGATATTGA	960
20	ACACGGAATA CCAAATTA AAAGGATACA CATGGCCTTC ATATGAACCG TGAACCTTTG	1020
	ATAACGTGGA AGTTCAAAGA AGGTAAAGTT TAAGAATAAA CTGACAAATT AATTTCTTTT	1080
	ATTTGGCCCA CTAATAAATT TGCTTTACTT TCTAACATGT CAAGTTGTGC CCTCTTAGTT	1140
25	GAATGATATT CATTTTTCAT CCCATAAGTT CAATTTGATT GTCATACCAC CCATGATGTT	1200
	CTGAAAAATG CTTGGCCATT CACAAAGTTT ATCTTAGTTC CTATGAACCT TATAAGAAGC	1260
30	TTTAATTTGA CATGTTATTT ATATTAGATG ATATAATCCA TGACCCAATA GACAAGTGTA	1320
	TTAATATTGT AACTTTGTAA TTGAGTGTGT CTACATCTTA TTCAATCATT TAAGGTCATT	1380
	AAAATAAATT ATTTTTGAC ATTCTAAAC TTTAAGCAGA ATAAATAGTT TATCAATTAT	1440
35	TAAAAACAAA AAACGACTTA TTTATAAATC AACAAACAA TTTAGATTGC TCCAACATAT	1500
	TTTTCCAAAT TAAATGCAGA AAATGCATAA TTTTATACTT GATCTTTATA GCTTATTTTT	1560
40	TTTAGCCTAA CCAACGAATA TTTGTAACT CACAACCTGA TTAAAAGGGA TTTACAACAA	1620
	GATATATATA AGTAGTGACA AATCTTGATT TTAAATATTT TAATTTGGAG GTCAAAATTT	1680
	TACCATAATC ATTTGTATTT ATAATTAAAT TTAAATATC TTATTTATAC ATATCTAGTA	1740
45	AACTTTTAAA TATACGTATA TACAAAATAT AAAATTATTG GCGTTCATAT TAGGTCAATA	1800
	AATCCTTAAC TATATCTGCC TTACCACTAG GAGAAAGTAA AAAACTCTTT ACCAAAAATA	1860
50	CATGTATTAT GTATACAAAA AGTCGATTAG ATTACCTAAA TAGAAATTGT ATAACGAGTA	1920
	AGTAAGTAGA AATATAAAAA AACTACAATA CTAAAAAAA TATGTTTTAC TTCAATTTTCG	1980
	AAACTAATGG GGTCTGAGTG AAATATTCTAG AAAGGGGAGG ACTAACAAAA GGGTCATAAT	2040
55	GTTTTTTTAT AAAAAGCCAC TAAATGAGG AAATCAAGAA TCAGAACATA CAAGAAGGCA	2100

	GCAGCTGAAG CAAAGTACCA TAATTTAATC AATGGAAAT AATTTCAAAG TTTTATCAAA	2160
	ACCCATTCTGA GGATCTTTTC CATCTTTCTC ACCTAAAGTT TCTTCAGGGG TAATTTTAC	2220
5	TAATTTTCATG TTAATTTCAA TTATTTTATAG CCTTTCGATT TCATTTTCCA ATATATCTGG	2280
	ATCATCTCCT TAGTTTTTAA TTTTATTTTT TATAATATCA AATATGGAAG AAAAATGACA	2340
10	CTGTAGAGC CATATGTAAG TATCATGTGA CAAATTTGCA AGGTGGTTGA GTGTATAAAA	2400
	TTCAAAAATT GAGAGATGGA GGGGGGGTGG GGAAGACAA TATTTAGAAA GAGTGTCTA	2460
	GGAGGTTATG GAGGACACGG ATGAGGGGTA GAAGGTTAGT TAGGTATTG AGTGTGTCT	2520
15	GGCTTATCCT TTCATACTAG TAGTCGTGGA ATTATTTGGG TAGTTTCTTG TTTGTTATT	2580
	TGATCTTTGT TATTCTATTT TCTGTTTCTT GTACTTCGAT TATTGTATTA TATATCTTGT	2640
20	CGTAGTTATT GTTCCTCGGT AAGAATGCTC TAGCATGCTT CCTTTAGTGT TTTATCATGC	2700
	CTTCTTTATA TTCGCGTTGC TTTGAAATGC TTTTACTTTA GCCGAGGGTC TATTAGAAAC	2760
	AATCTCTCTA TCTCGTAAGG TAGGGGTAAA GTCCTCACCA CACTCCACTT GTGGGATTAC	2820
25	ATTGTGTTTG TTGTTGTAAA TCAATTATGT ATACATAATA AGTGGATTTT TTACAACACA	2880
	AATACATGGT CAAGGGCAAA GTTCTGAACA CATAAAGGGT TCATTATATG TCCAGGGATA	2940
30	TGATAAAAAT TGTTTCTTTG TGAAAGTTAT ATAAGATTG TTATGGCTTT TGCTGGAAC	3000
	ATAATAAGTT ATAATGCTGA GATAGCTACT GAAGTTTGT TTTCTAGCC TTTTAAATGT	3060
	ACCAATAATA GATTCCGTAT CGAACGAGTA TGTTTTGATT ACCTGGTCAT GATGTTTCTA	3120
35	TTTTTTACAT TTTTTTGGTG TTGAACTGCA ATTGAAAATG TTGTATCCTA TGAGACGGAT	3180
	AGTTGAGAAT GTGTTCTTTG TATGGACCTT GAGAAGCTCA AACGCTACTC CAATAATTTT	3240
40	TATGAATTCA AATTCAGTTT ATGGCTACCA GTCAGTCCAG AAATTAGGAT ATGCTGCATA	3300
	TACTTGTTCA ATTATACTGT AAAATTTCTT AAGTTCTCAA GATATCCATG TAACCTCGAG	3360
	AATTTCTTTG ACAGGCTTCT AGAAATAAGA TATGTTTTCC TTCTCAACAT AGTACTGGAC	3420
45	TGAAGTTTGG ATCTCAGGAA CGGTCTTGGG ATATTTCTTC CACCCCAAAA TCAAGAGTTA	3480
	GAAAAGATGA AAGGGTATGT TTGATAATTT ATATGGTTGC ATGGATAGTA TATAAATAGT	3540
50	TGGAAAACCT CTGGACTGGT GCTCATGGCA TATTTGATCT GTGCACCGTG TGGAGATGTC	3600
	AAACATGTGT TACTTCGTTT CGCCAATTTA TAATACCTTA ACTTGGGAAA GACAGCTCTT	3660
	TACTCCTGTG GGCATTTGTT ATTGAATTA CAATCTTTAT GAGCATGGTG TTTTCACATT	3720
55	ATCAACTTCT TTCATGTGGT ATATAACAGT TTTTAGCTCC GTTAATACCT TTCTTCTTTT	3780
	TGATATAAAC TAACTGTGGT GCATTGCTTG CATGAAGCAC AGTTCAGCTA TTTCCGCTGT	3840

	TTTGACCGAT GACGACAATT CGACAATGGC ACCCCTAGAG GAAGATGTCA AGACTGAAAA	3900
5	TATTGGCCTC CTAAATTTGG ATCCAACCTT GGAACCTTAT CTAGATCACT TCAGACACAG	3960
	AATGAAGAGA TATGTGGATC AGAAAATGCT CATTGAAAAA TATGAGGGAC CCCTTGAGGA	4020
	ATTTGCTCAA GGTAACAGCC AAAAGTTGTG CTTTAGGCAG TTTGACCTTA TTTTGAAGA	4080
10	TGAATTGTTT ATACCTACTT TGACTTTGCT AGAGAATTTT GCATACCGGG GAGTAAGTAG	4140
	TGGCTCCATT TAGGTGGCAC CTGGCCATT TTTTGATCTT TTA AAAAGCT GTTTGATTGG	4200
15	GTCTTCAAAA AAGTAGACAA GGTTTTTGA GAAGTGACAC ACCCCCGGAG TGTCAGTGGC	4260
	AAAGCAAAGA TTTTCACTAA GGAGATTCAA AATATAAAAA AAGTATAGAC ATAAAGAAGC	4320
	TGAGGGGATT CAACATGTAC TATACAAGCA TCAAATATAG TCTTAAAGCA ATTTGTAGA	4380
20	AATAAGAAA GTCTTCCTTC TGTGCTTCA CAATTTCTT CTATTATCAT GAGTTACTCT	4440
	TTCTGTTTGA AATAGCTTCC TTAATATTAA ATTCATGATA CTTTGTGGA GATTAGCAG	4500
25	TTTTTCTTG TGTAAGTGC TCTCTTTTT TGCAGGTTAT TTA AAAATTG GATTCAACAG	4560
	GGAAGATGGT TGCATAGTCT ATCGTGAATG GGCTCCTGCT GCTCAGTAGG TCCTCGTCTA	4620
	CTACAAAATA GTAGTTTCCA TCATCATAAC AGATTTTCCT ATTAAAGCAT GATGTTGCAG	4680
30	CATCATTGGC TTTCTTACAT GTTCTAATTG CTATTAAGGT TATGCTTCTA ATTAACCTAT	4740
	CCACAATGCA GGAAGCAGA AGTTATTGGC GATTTCAATG GATGGAACGG TTCTAACCAC	4800
35	ATGATGGAGA AGGACCAGTT TGGTGTGTTG AGTATTAGAA TTCCTGATGT TGACAGTAAG	4860
	CCAGTCATTC CACACAATC CAGAGTTAAG TTTGTTTCA AACATGGTAA TGGAGTGTGG	4920
	GTAGATCGTA TCCCTGCTTG GATAAAGTAT GCCACTGCAG ACGCCACAAA GTTTCAGCA	4980
40	CCATATGATG GTGTCTACTG GGACCCACCA CCTTCAGAAA GGTTTTGTTA TTCATACCTT	5040
	GAAGCTGAAT TTTGAACACC ATCATCACAG GCATTTTCGAT TCATGTTCTT ACTAGTCTTG	5100
45	TTATGTAAGA CATTTTGAAA TGCAAAAGTT AAAATAATTG TGTCTTACT AATTTGACT	5160
	TGATCCCATC CTCTTCCCT TAACAAAATG AGTCAATTCT ATAAGTGCTT GAGAACTTAC	5220
	TACTTCAGCA ATTAAACAGG TACCACTTCA AATACCCTCG CCCTCCCAA CCCCAGAGCC	5280
50	CACGAATCTA TGAAGCACAT GTCGGCATGA GCAGCTCTGA GCCACGTGTA AATTCGTATC	5340
	GTGAGTTTGC AGATGATGTT TTACCTCGGA TTAAGGCAAA TAACTATAAT ACTGTCCAGT	5400
55	TGATGGCCAT AATGGAACAT TCTTACTATG GATCATTTGG ATATCATGTT ACAAACCTTT	5460
	TTGCTGTGAG CAGTAGATAT GGAAACCCGG AGGACCTAAA GTATCTGATA GATAAAGCAC	5520

	ATAGCTTGGG	TTTACAGGTT	CTGGTGGATG	TAGTTCACAG	TCATGCAAGC	AATAATGTCA	5580
	CTGATGGCCT	CAATGGCTTT	GATATTGGCC	AAGGTTCTCA	AGAATCCTAC	TTTCATGCTG	5640
5	GAGAGCGAGG	GTACCATAAG	TTGTGGGATA	GCAGGCTGTT	CAACTATGCC	AATTGGGAGG	5700
	TTCTTCGTTT	CCTTCTTTCC	AACTTGAGGT	GGTGGCTAGA	AGAGTATAAC	TTTGACGGAT	5760
	TTGATTTGA	TGGAATAACT	TCTATGCTGT	ATGTTTCATCA	TGGAATCAAT	ATGGGATTTA	5820
10	CAGGAAACTA	TAATGAGTAT	TTCAGCGAGG	CTACAGATGT	TGATGCTGTG	GTCTATTTAA	5880
	TGTTGGCCAA	TAATCTGATT	CACAAGATTT	TCCCAGATGC	AACTGTTATT	GCCGAAGATG	5940
15	TTTCTGGTAT	GCCGGGCCTT	GGCCGGCCTG	TTTCTGAGGG	AGGAATTGGT	TTTGTTTACC	6000
	GCCTGGCAAT	GGCAATCCCA	GATAAGTGGA	TAGATTATTT	AAAGAATAAG	AATGATGAAG	6060
	ATTGGTCCAT	GAAGGAAGTA	ACATCGAGTT	TGACAAATAG	GAGATATACA	GAGAAGTGTA	6120
20	TAGCATATGC	GGAGACCCAT	GATCAGGTAT	TTTAAATTTA	TTTCTACAAC	TAAATAATTC	6180
	TCAGAACAA	TGTTAGATAG	AATCCAAATA	TATACGTCCT	GAAAGTATAA	AAGTACTTAT	6240
25	TTTCGCCATG	GGCCTTCAGA	ATATTGGTAG	CCGCTGAATA	TCATGATAAG	TTATTTATCC	6300
	AGTGACATTT	TTATGTTTAC	TCCTATTATG	TCTGCTGGAT	ACAGTCTATT	GTTGGTGACA	6360
	AGACCATTC	ATTTCTCCTA	ATGGACAAAG	AGATGTATTC	TGGCATGTCT	TGCTTGACAG	6420
30	ATGCTTCTCC	TGTTGTTGAT	CGAGGAATTG	CGCTTCACAA	GGTTTGTCTG	TTTCTATTGC	6480
	ATTTTAAGGT	TCATATAGGT	TAGCCACGGA	AAATCTCACT	CTTTGTGAGG	TAACCAGGGT	6540
35	TCTGATGGAT	TATTCAATTT	TCTCGTTTAT	CATTTGTTTA	TTCTTTTCAT	GCATTGTGTT	6600
	TCTTTTTCAA	TATCCCTCTT	ATTTGGAGGT	AATTTTCTC	ATCTATTAC	TTTAGCTTC	6660
	TAACCACAGA	TGATCCATTT	TTTCACAATG	GCCTTGGGAG	GAGAGGGGTA	CCTCAATTTT	6720
40	ATGGGTAACG	AGGTATGTCT	TACATCTTTA	GATATTTTGT	GATAATTACA	ATTAGTTTGG	6780
	CTTACTTGAA	CAAGATTCAT	TCCTCAAAAT	GACCTGAACT	GTTGAACATC	AAAGGGGTTG	6840
45	AAACATAGAG	GAAAACAACA	TGATGAATGT	TTCCATTGTC	TAGGGATTTC	TATTATGTTG	6900
	CTGAGAACAA	ATGTCATCTT	AAAAAAAACA	TGTTTACTT	TTTGTAGTA	TAGAAGATTA	6960
	CTGTATAGAG	TTTGCAAGTG	TGTCTGTTTT	GGAGTAATG	TGAAATGTTT	GATGAACTTG	7020
50	TACAGTTTGG	CCATCCTGAG	TGGATTGACT	TCCCTAGAGA	GGGCAATAAT	TGGAGTTATG	7080
	ACAAATGTAG	ACGCCAGTGG	AACCTCGCGG	ATAGCGAACA	CTTGAGATAC	AAGGTTCAAG	7140
55	TATTTTGAAT	CGCAGCTTGT	TAAATAATCT	AGTAATTTT	AGATTGCTTA	CTTGGAAGTC	7200
	TACTTGGTTC	TGGGGATGAT	AGCTCATTTT	ATCTTGTCT	ACTTATTTT	CAACCGAATT	7260

	TCTGATTTTT	GTTCGAGAT	CCAAGTATTA	GATTCATTTA	CACTTATTAC	CGCCTCATTT	7320
	CTACCACTAA	GGCCTTGATG	AGCAGCTTAA	GTTGATTCTT	TGAAGCTATA	GTTTCAGGCT	7380
5	ACCAATCCAC	AGCCTGCTAT	ATTTGTTGGA	TACTTACCTT	TTCTTTACAA	TGAAGTGATA	7440
	CTAATTGAAA	TGGTCTAAAT	CTGATATCTA	TATTTCTCCG	TCTTTCCTCC	CCCTCATGAT	7500
10	GAAATGCAGT	TTATGAATGC	ATTTGATAGA	GCTATGAATT	CGCTCGATGA	AAAGTTCTCA	7560
	TTCCTCGCAT	CAGGAAAACA	GATAGTAAGC	AGCATGGATG	ATGATAATAA	GGTAAAATCA	7620
	TCTAAAGTTG	AAAGTGTG	GTTTATGAAG	TGCTTTAATT	CTATCCAAGG	ACAAGTAGAA	7680
15	ACCTTTTTAC	CTTCCATTTT	TTGATGATGG	ATTTCATATT	ATTTAATCCA	ATAGCTGGTC	7740
	AAATTCGGTA	ATAGCTGTAC	TGATTAGTTA	CTTCACTTTG	CAGGTTGTTG	TGTTTGAACG	7800
20	TGGTGACCTG	GTATTTGTAT	TCAACTTCCA	CCCAAAGAAC	ACATACGAAG	GGTATATATG	7860
	TTTTACTTAT	CCATGAAATT	ATTGCTCTGC	TTGTTTTTAA	TGTAAGTGAAC	AAGTTTTATG	7920
	GAGAAGTAAC	TGAAACAAAT	CATTTTCACA	TTGTCTAATT	TAAGTCTTTT	TTCTGATCCT	7980
25	CGCATGACGA	AAACAGGTAT	AAAGTTGGAT	GTGACTTGCC	AGGGAAGTAC	AGAGTTGCAC	8040
	TGGACAGTGA	TGCTTGGGAA	TTGGTGGCC	ATGGAAGAGT	AAGGATTTGC	TTGAATAACT	8100
30	TTTGATAATA	AGATAACAGA	TGTAGGTGAC	AGTTCTCTCA	CCAAAAAGAA	CTGTAATTGT	8160
	CTCATCCATC	TTTAGTTGTA	TAAGATATCC	GACTGTCTGA	GTTCCGAAGT	GTTTGAGCCT	8220
	CCTGCCCTCC	CCCTGCGTTG	TTTAGCTAAT	TCAAAAAGGA	GAAAACTGTT	TATTGATGAT	8280
35	CTTTGTCTTC	ATGCTGACAT	ACAATCTGTT	CTCATGACAG	ACTGGTCATG	ATGTTGACCA	8340
	TTTCACATCA	CCAGAAGGAA	TACCTGGAGT	TCCAGAAACA	AATTTCAATG	GTCGTCCAAA	8400
40	TTCCTTCAAA	GTGCTGTCTC	CTGCGCGAAC	ATGTGTGGTA	CAGTTCTTGC	CGTGTGACCT	8460
	CCCTTTTTAT	TGTGGTTTTG	TTCATAGTTA	TTGAATGCG	ATAGAAGTTA	ACTATTGATT	8520
	ACCGCCACAA	TCGCCAGTTA	AGTCCTCTGA	ACTACTAATT	TGAAAGGTAG	GAATAGCCGT	8580
45	AATAAGGTCT	ACTTTTGGCA	TCTTACTGTT	ACAAAACAAA	AGGATGCCAA	AAAAATTCTT	8640
	CTCTATCCTC	TTTTTCCCTA	AACCAGTGCA	TGTAGCTTGC	ACCTGCATAA	ACTTAGGTAA	8700
50	ATGATCAAAA	ATGAAGTTGA	TGGGAACCTA	AAACCGCCCT	GAAGTAAAGC	TAGGAATAGT	8760
	CATATAATGT	CCACCTTTGG	TGTCTGCGCT	AACATCAACA	ACAACATACC	TCGTGTAGTC	8820
	CCACAAAGTG	GTTTCAGGGG	GAGGGTAGAG	TGTATGCAAA	ACTTACTCCT	ATCTCAGAGG	8880
55	TAGAGAGGAT	TTTTTCAATA	GACCCCTTGGC	TCAAGAAAAA	AAGTCCAAAA	AGAAGTAACA	8940

	GAAGTGAAAG CAACATGTGT AGCTAAAGCG ACCCAACTTG TTTGGGACTG AAGTAGTTGT	9000
	TGTTGTTGAA ACAGTGCATG TAGATGAACA CATGTCAGAA AATGGACAAC ACAGTTATTT	9060
5	TGTGCAAGTC AAAAAAATGT ACTACTATTT CTTTGTGCAG CTTTATGTAT AGAAAAGTTA	9120
	AATAACTAAT GAATTTTGCT AGCAGAAAAA TAGCTTGGAG AGAAATTTTT TATATTGAAC	9180
10	TAAGCTAACT ATATTCATCT TTCTTTTTGC TTCTTCTTCT CCTGTTTTGT GAAGGCTTAT	9240
	TACAGAGTTG ATGAACGCAT GTCAGAACT GAAGATTACC AGACAGACAT TTGTAGTGAG	9300
	CTACTACCAA CAGCCAATAT CGAGGAGAGT GACGAGAAAC TTAAAGATTC GTTATCTACA	9360
15	AATATCAGTA ACATTGACGA ACGCATGTCA GAAACTGAAG TTTACCAGAC AGACATTTCT	9420
	AGTGAGCTAC TACCAACAGC CAATATTGAG GAGAGTGACG AGAACTTAA AGATTCGTTA	9480
20	TCTACAAATA TCAGTAACAT TGATCAGACT GTTGATGTTT CTGTTGAGGA GAGAGACAAG	9540
	GAACTTAAAG ATTCACCGTC TGTAAGCATC ATTAGTGATG TTGTTCCAGC TGAATGGGAT	9600
	GATTCAGATG CAAACGTCTG GGGTGAGGAC TAGTCAGATG ATTGATCGAC CCTTCTACCG	9660
25	ATTGGTGATC GCTATCCTTG CTCTCTGAGA AATAGGTGAG GCGAAACAAA AAATAATTTG	9720
	CATGATAAAA AGTCTGATTT TATGATCGCT ATCCTCGCTC TCTGAGAAAG AAGCGAAACA	9780
	AAGGCGACTC CTGGACTCGA ATCTATAAGA TAACAAAGGC GACTCCTGGG ACTCGAATCT	9840
30	ATAAGATAAC AAAGGCAATT CCAAGACTTG AATCTATAAA AAATTTAGTT AAGAATGATT	9900
	AACGTCCGAT CCTAATTCGA ATCGAGGCAT CTTACCACTC CATTGATAAT TATATAAGTC	9960
35	AATAAGTCAT ATAAAGTATT AAAAATAAAA TTGACTTGAT CGGTCTATCA AAAATAGATA	10020
	AATTGTGTTT ATATGTAACA TTTTGTGTGT CACAATTAGC TTAATTACAT CTTTCATGTG	10080
	CAATAACAAA GAAATGATAG GAATTTAGAG ATTCCAATTT TTTTGTGCCC ACAATTAAC	10140
40	TAATTACATC TTTCATTTGC AATAACAAAG AAATGATAGG AATTTAGAGA TCCAGTGTCA	10200
	ATACACAACC TAGGCCAACA TCGAAAGCAT AACTGTAAAC TCATGCATGA AGAAATCAGT	10260
45	CGTAAAAATG AATAAATGCG ACATAAAAAAC AAATTGCATG TATCATTAAT GTGACTTAAC	10320
	TACAAGTAAA AATAAATTTA ACAAATGTAA CTTAACTACA AGTAAAAATA AATTGCTTCT	10380
	ATCATTAACA AACAAACAGA ATTAAAAAGA AAAAAACATA CTAAATCTTA CCGTCATTCTG	10440
50	ATAAAAAAAA ATACCAAATT CATAATGCAA GGAAAACGAA ACGCGTCCTG ATCGGGTATC	10500
	AACGATGAAA TGGACCAGTT GGATCGACTG CCTGCACAAC GTTAGGTATG CCAAAAAAAA	10560
55	GAACACGATC CTTTGCACCC GTTCGATGAT TATCAGTATG TTCACAAAAA AACTTAAGT	10620
	TCATCCAGT GTACAACAGC CCCAACATCT GCCCAAGTA ACAAAAAACA ACCAATTTAT	10680

CTTATTCTTA TCTGCCACAA AATAATCGGT TTCACACTAT TCTCTTGTTA TACAAAATTG 10740
ACAAGTAGGA AGGAGAGGAG TCATCCAAAT AAACGGTGCA CGTTCTTTGA GAAAAGTCTT 10800
5 ATTTTTCGTA AGATCCAATT TCAACAAACT TTTCTTCAAG TCAAAATTCC TGATAGTGTA 10860
TCTCCTCTCG ACGACCTCTT GCATTGAACG ATCTCCGCTT ATCATGAAAA GTTGCTTGGA 10920
10 TAACAAGTAT TGCAAGGGGG GGACAGTAGC TATTAAAGTTA GTCGGCCCAA GGAAATGGAG 10980
GAGTGATAGT CTCGAATATT ATTCACCTCT TTAGCATTAC CCGGTCTGGC TTTAAGGAGT 11040
TACGTCTTTT ACGCTCGCCA ATTTCTTTTT TTAGAATGGT TGGTGTCAAA ATCGCGAGTT 11100
15 GTGGAAGGTT CAAGTTACTC GATTCTGTAT TTTCAAGTAT GAGTGGTGAG AGAGATTCTGA 11160
TATTTTCACG AGGTGTATTC GAGGTCTAGT AGAACGAAGG GTGTCACTAA TGAAAGTTTC 11220
20 AAGAGTTCAT CATCATCTTC TTCTAGTAGA TTTTCGCTTT CAAATGAGTA TGAAAATTCT 11280
TCCTCTTTTC TATTGATTTT CTTTCTGTT TTCTTCATTG TTGTGGTTGT TATTGAAAAG 11340
AAAGAAAATT TATAACAGAA AAAGATGTCA AAAAAAGGT AAAATGAAAG AGTATCATAT 11400
25 ACTTAAAGAG TTGCGTAGAG ATAAGTCAAA AGAAACAGAA TTATAGTAAT TTCAGCTAAG 11460
TTAGAATTCT 11469

30 (2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

35 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

40 (A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

50 GGAATTCAG TCGCAGTCTA CATTAC

26

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

55 (A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

56

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

15 CGGGATCCAG AGGCATTAAG ATTTCTGG

28

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

35 CGGGATCCAA AGAAATTCTC GAGGTACAT GG

32

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CGGGATCCGG GGTAATTTT ACTAATTTCA TG

32

(2) INFORMATION FOR SEQ ID NO: 34:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

15 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CGGGATCCCG TATGTCTCAC TGTGTTTGTG GC

32

25

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

30 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

35 (A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

45

CGGGATCCCC CTACATACAT ATATCAGATT AG

32

(2) INFORMATION FOR SEQ ID NO: 36:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

10 CCATCGATAC TTAAAGTGAT TTGATGGC 28

(2) INFORMATION FOR SEQ ID NO: 37:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

25 (iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

30 CGGGATCCTG TTCTGATTCT TGATTTC 28

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2122 base pairs

(B) TYPE: nucleic acid

40 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

45 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

55 GTATGTCTCA CTGTGTTTGT GGCTGTGTGT GTTTTTTCT CTGTCTTTT GTGTTTGTG 60

TAATTGGGGC TCTTTAAAGT TGGTATTGTG TATACCCTTT TGAGTATAGT CTTTGAGGAA 120

	GCAAAATGAT	GAATCTTGAT	TGACATTAGT	AAGGGTTGTA	ACTTTTTGAA	GTTTGGTTAG	180
	GTGTAATTGA	GTTTGGCTTG	TGTGTCTGTG	TGTCGAGGTT	ATTTTTTTGG	TTTGTGTTAT	240
5	TGGGGATTCT	TAAAAGTTGG	TATTGTGTAT	ACCCTTTTGA	GTATAGTCTT	TGAGGAAGCA	300
	AAAATGATGA	ATCTTGATTG	GCATTAGTAA	AGGTTGTAGC	TTTTTGAAGT	GTGGTTAGGT	360
	GTAATTGAGT	TTGGCTTGTG	TGTCTGTGTG	TTTTGGAATC	CTGATGTGTG	TCAAGTCCTG	420
10	ATATGGGTCG	AGGTTCTTTC	TTTGGTTTGT	GTAATTGGGG	GTTCTTAAAA	GTTGGTATTA	480
	TGTACCTTTT	TAAGAATAGT	GTCTGAGAAA	GCAAAATCGA	TGAATTTTGA	TTGACAGCAT	540
15	ATTCTTTGAG	AAAGCAAAAA	ATGGTGAGTT	TTCATGGAGA	AACTTGATTG	ACATTACTAA	600
	AGGTAGCAAC	TTTTTCAACT	CCTGATATGG	GTCAAGGTTT	TTTGTTTGGT	TTGTGTAATT	660
	TGGGGTTCTT	TGAAGTTTTG	AGAAAGAAAA	ATTATGATTT	TTCATGGAGA	AATTTGATTT	720
20	ACATTAATAA	AGGTAGTAGC	TTTTTAAAGT	GTGGTCAGCT	GTAATGAGTT	CAGCTTGGTT	780
	TAAAGGGGCC	CTACATATGG	TGCTTCTCTG	TGAGATATTT	GTTGCTCCAC	CATACGAGTT	840
25	ATAAGAATCA	TAGTGTTAGG	ATCTTTTTTC	TTTTTTTTTT	CATTTTTCAC	TTGACTAGCT	900
	ACTAGAGGAG	TGATCTTGAC	GGCGGAAAAT	CTTAGAAAGG	GGAAGGTTGT	TTGCATCAAC	960
	TGGTGTTATA	TGTGCAAGGA	GACGGGAGAT	GATGTAGATC	ATCTTCTTCT	TCATTGTGGT	1020
30	CTTTCATGA	GGTTATGATG	TGATATGTTT	GAATGGTTTG	GTACTTCTTG	GCTATGCCAA	1080
	GAACTGTGAA	AGAATTGATA	TTCAGTTGGA	AGTGTGGAGT	TGGAAGAGTG	GAAGAATTGA	1140
35	CACCTGGTTC	CATTAGCTTT	AATGTGGGTG	GTGTGGAGAG	AGAGAGAAAT	AGGAGAGCTT	1200
	TTGAGGGGGT	AGAGTTGAGC	TTTCCTCAGT	TGAGAAGTAG	CCTTTGATAT	CTTTTTTTTT	1260
	TTTTTTTGTA	CACCCATAGA	ATTCCCAATT	GTATAGAAGA	TTGGGTGGAG	TTTGTAGAGA	1320
40	ATCATCTTTT	GTAGTAGATT	CTTACCTTT	TGGTATATCC	ATTGTATACA	GCCAGGCCTT	1380
	TGACTATGTT	TATGAATGAA	TATACATTAC	TTGAAAAAAA	AAGAAGTGAA	GCCAGTCTGT	1440
45	TGTACCTTTG	TAGACAAATG	TGTTGCAGCA	TCTTGATAAT	TCCCTGAAAA	TTGTCTCCCT	1500
	GAAGGAATAG	TTTGGTTGAT	ATTGATTATT	TCTTGGTTTG	TTTAATTTCGG	TGTTCTTGAA	1560
	GGCCATTTTA	AATCCTTTGA	CATTGTTAAA	GGTGTTTACA	AGTGTGGTTC	TGGGTTTAAA	1620
50	AGCACCTCTT	GTATGGTGCT	TTCTGGAGTG	ATCTTCTTTC	CTCCAAAAGA	GAAGTTGCAA	1680
	GAATCAGTGT	GTGTACTTTT	TTCTCTTGTA	TGATCAGATC	TTTTTTCAAT	TTTTCCGTTT	1740
55	TAGTTGATTT	ATCCATATAG	TGAAAGTTGG	TGTCATAGTT	GCTGTTTGTG	GACTTCCTGT	1800
	AAAAGTTTTT	TGATATACTT	AAAAAATTGT	CACACAGAAG	AAAGAGTTTT	TTACCATTAC	1860

TTAAGCTAGA TGGGACTGTT TGATTCTTAG ACCAAATAAT GAACCTTTT GTTCTCTTAA 1920
CGTGTA CTG AAATAGTTG GTAAAATTGT GATAGGAAAA AAGATAATTC TTGATTGCTT 1980
5 TTGGAGCATC ACTTCTAATC ATAAAAGTCT TTGCTCTCTT CAACCATGAA TGATAAATTG 2040
GACACTTATG TGGCCCTAAG TTGCTCTCAG TAGTGGTCTT TAATTGTGGA GATATAACTA 2100
10 ATCTGATATA TGTATGTAGG GA 2122

CLAIMS

1. A method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron of a class A potato starch branching enzyme in an antisense orientation, optionally together with a nucleotide sequence which codes, partially or completely, for an intron of a class B starch branching enzyme in an antisense or sense orientation; and wherein the nucleotide sequence does not contain a sequence that is antisense to an exon sequence normally associated with the intron.
2. A method according to claim 1 wherein starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.
3. A method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron of a class A starch branching enzyme in an antisense orientation, optionally together with a nucleotide sequence which codes, partially or completely, for an intron of a class B starch branching enzyme in an antisense or sense orientation; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.
4. A method according to claim 3 wherein the nucleotide sequence does not contain a sequence that is antisense to an exon sequence normally associated with the intron.
5. A method according to any one of the preceding claims wherein the enzymatic activity is reduced or eliminated.

6. A method according to any one of the preceding claims wherein the nucleotide sequence codes for at least substantially all of at least one intron in an antisense orientation.
- 5 7. A method according to any one of the preceding claims wherein the nucleotide sequence codes for all of at least one intron in an antisense orientation.
8. A method according to any one of the preceding claims wherein the
10 nucleotide sequence comprises the complement of SEQ. ID. No. 38, or a fragment thereof.
9. A method according to any one of the preceding claims wherein the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ.I.D.
15 No. 14 or a variant, derivative or homologue thereof.
10. An antisense sequence comprising the nucleotide sequence as defined in claim 8 or a variant, derivative or homologue thereof.
- 20 11. A promoter having a sequence shown as SEQ.I.D. No. 14, or a variant, derivative or homologue thereof.
12. A promoter according to claim 11 in combination with a gene of interest ("GOI").
25
13. A construct capable of comprising or expressing the invention according to any one of claims 10 to 12.
14. A vector comprising or expressing the invention according to any one of
30 claims 10 to 13.

15. A combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in antisense orientation; wherein the intron is an intron that is associated with a genomic gene encoding an enzyme corresponding to the recombinant enzyme; and wherein the second nucleotide sequence does not contain a sequence that is antisense to an exon sequence normally associated with the intron.

16. A cell, tissue or organ comprising or expressing the invention according to any one of claims 10 to 15.

10

17. A transgenic starch producing organism comprising or expressing the invention according to any one of claims 10 to 16.

18. A transgenic starch producing organism according to claim 17 wherein the organism is a plant.

15

19. A starch obtained from the invention according to any one of the preceding claims.

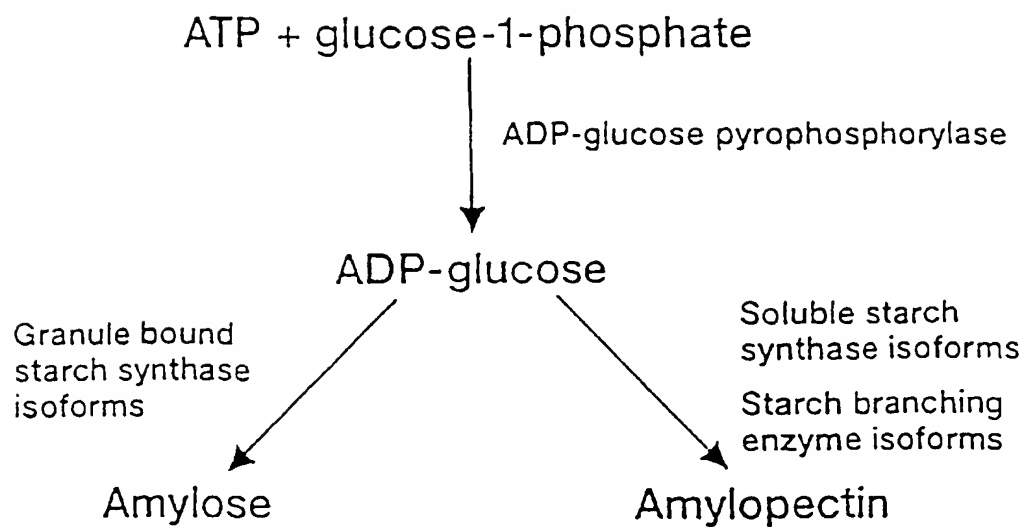
20

20. A nucleotide sequence that is antisense to an intron of class A SBE.

21. A method for modifying starch production in an organism, comprising transforming the organism with a transgene capable of expressing an antisense intron sequence relating to class A SBE and a transgene capable of expressing an antisense intron sequence relating to class B SBE, thereby reducing or eliminating endogenous class A and class B production, and a further sequence encoding a SBE from a heterologous source.

25

1 / 27



Reducing end



Reducing end

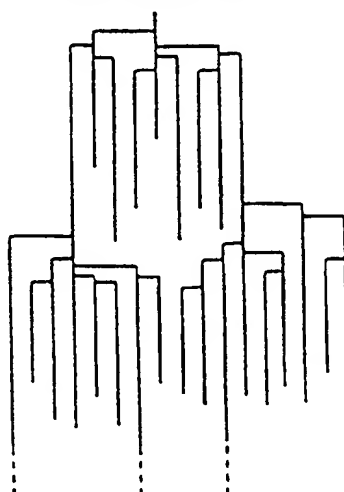


FIG. 1

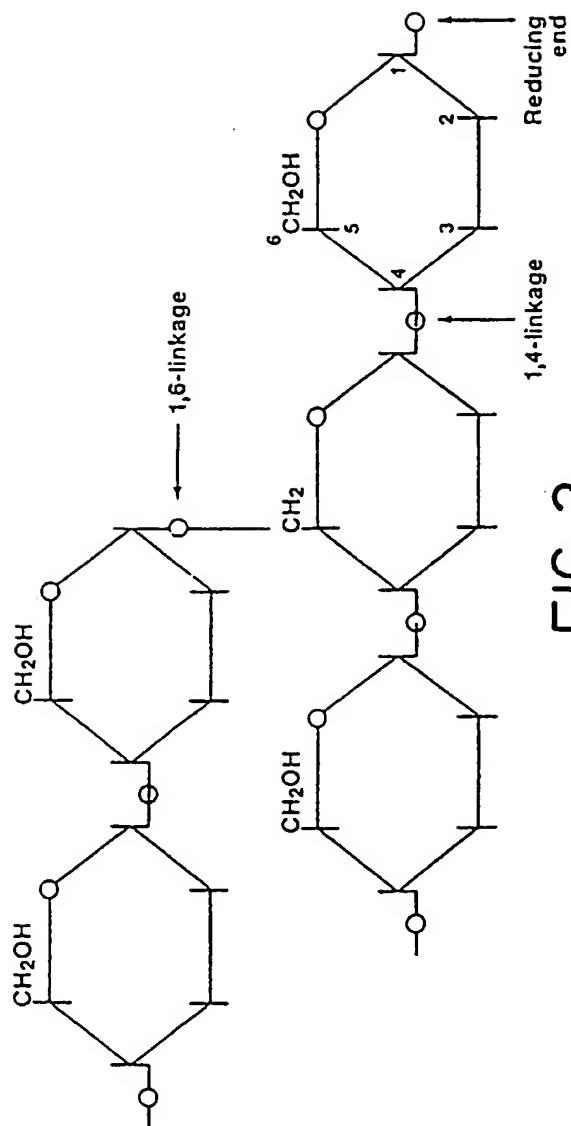


FIG. 2

3 / 27

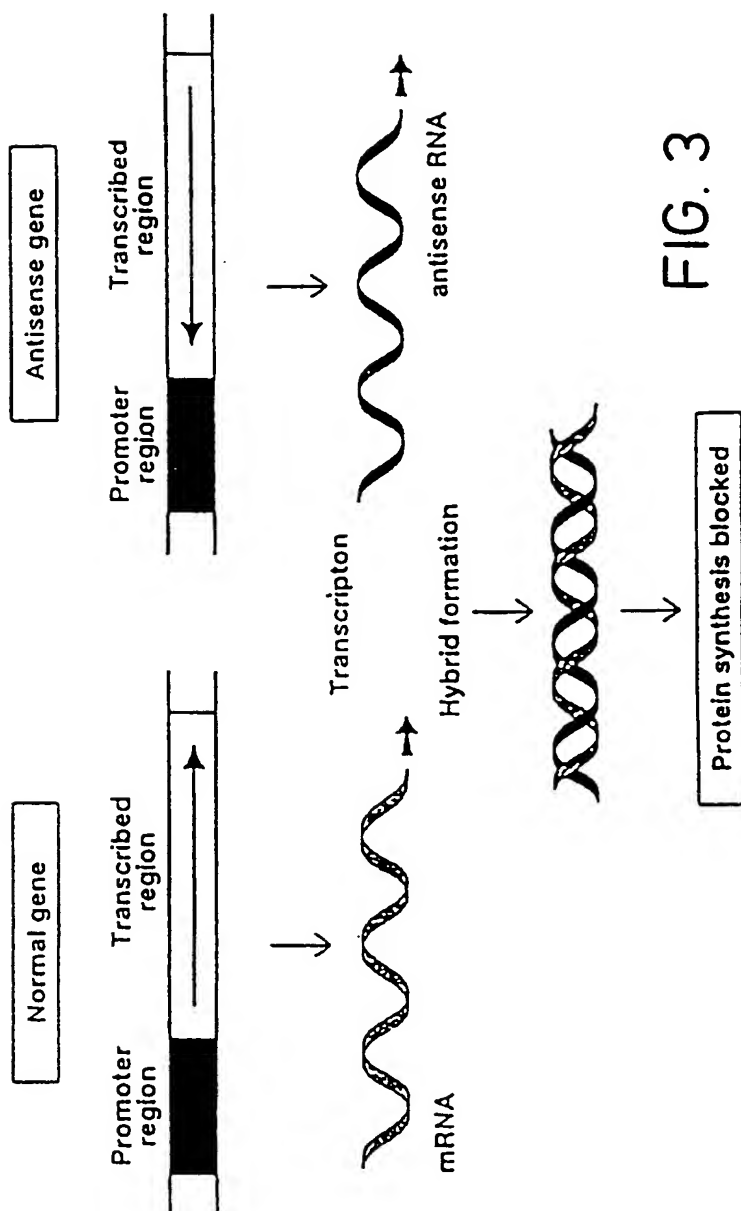


FIG. 3

4 / 27

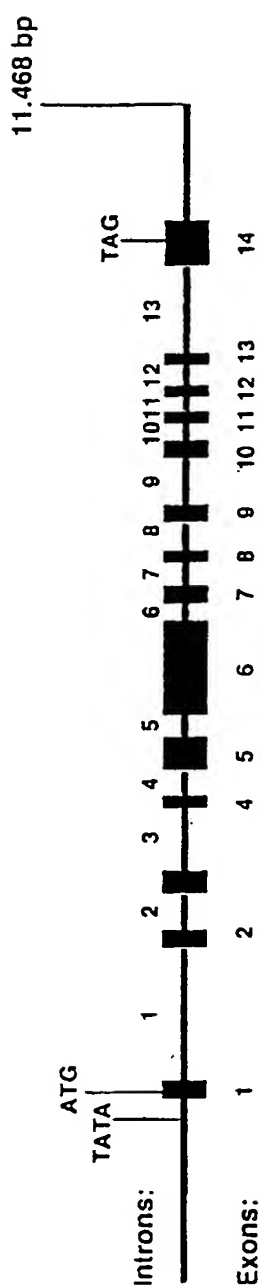


FIG. 4

5 / 27

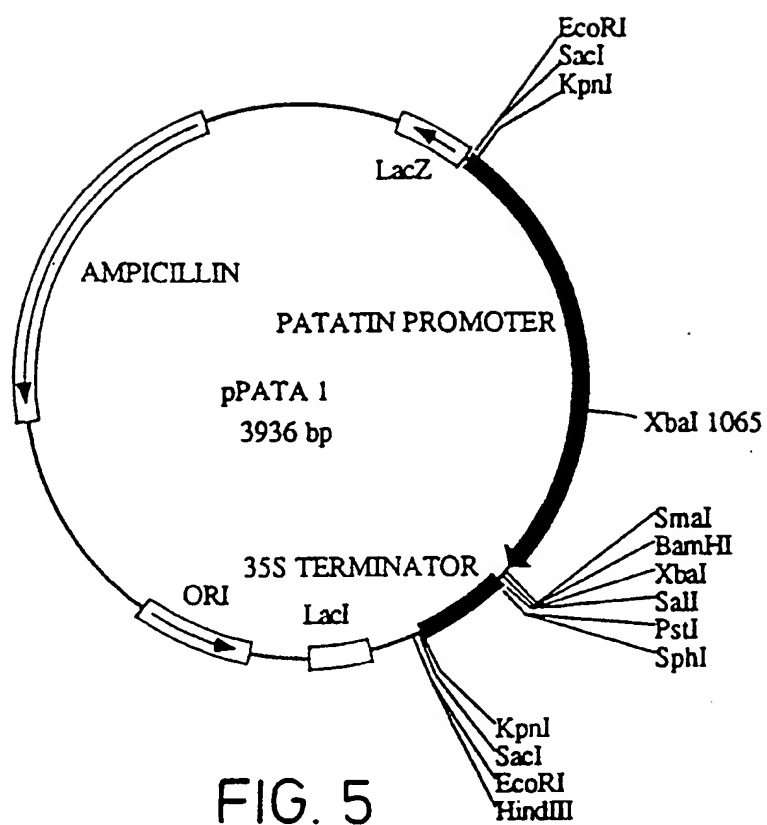


FIG. 5

6 / 27

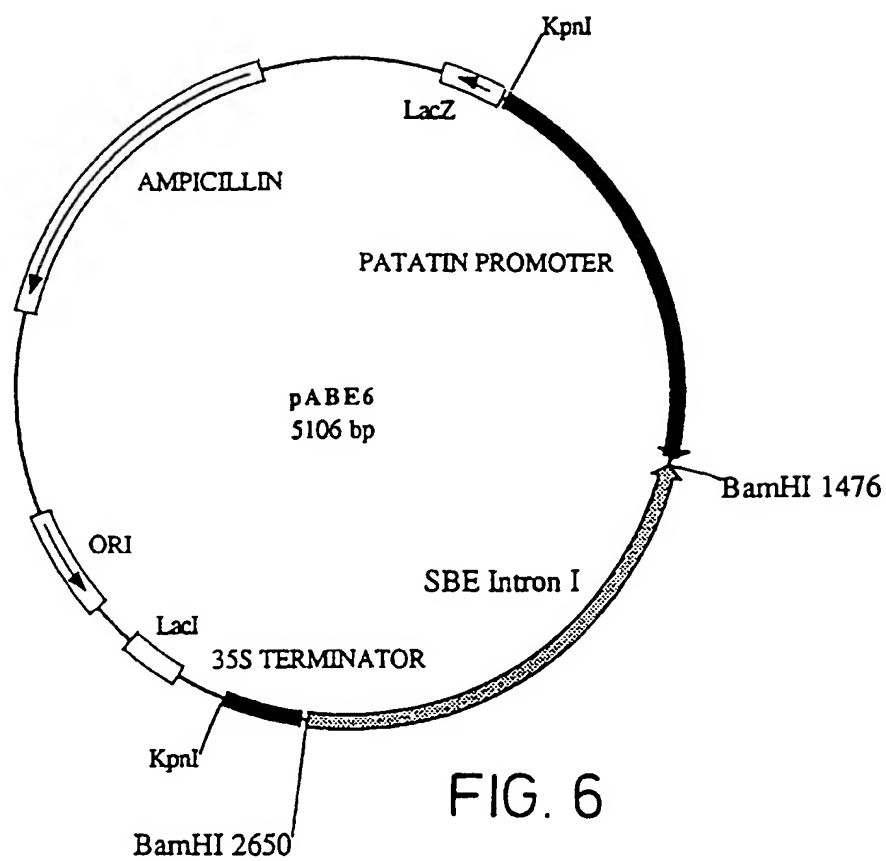


FIG. 6

7 / 27

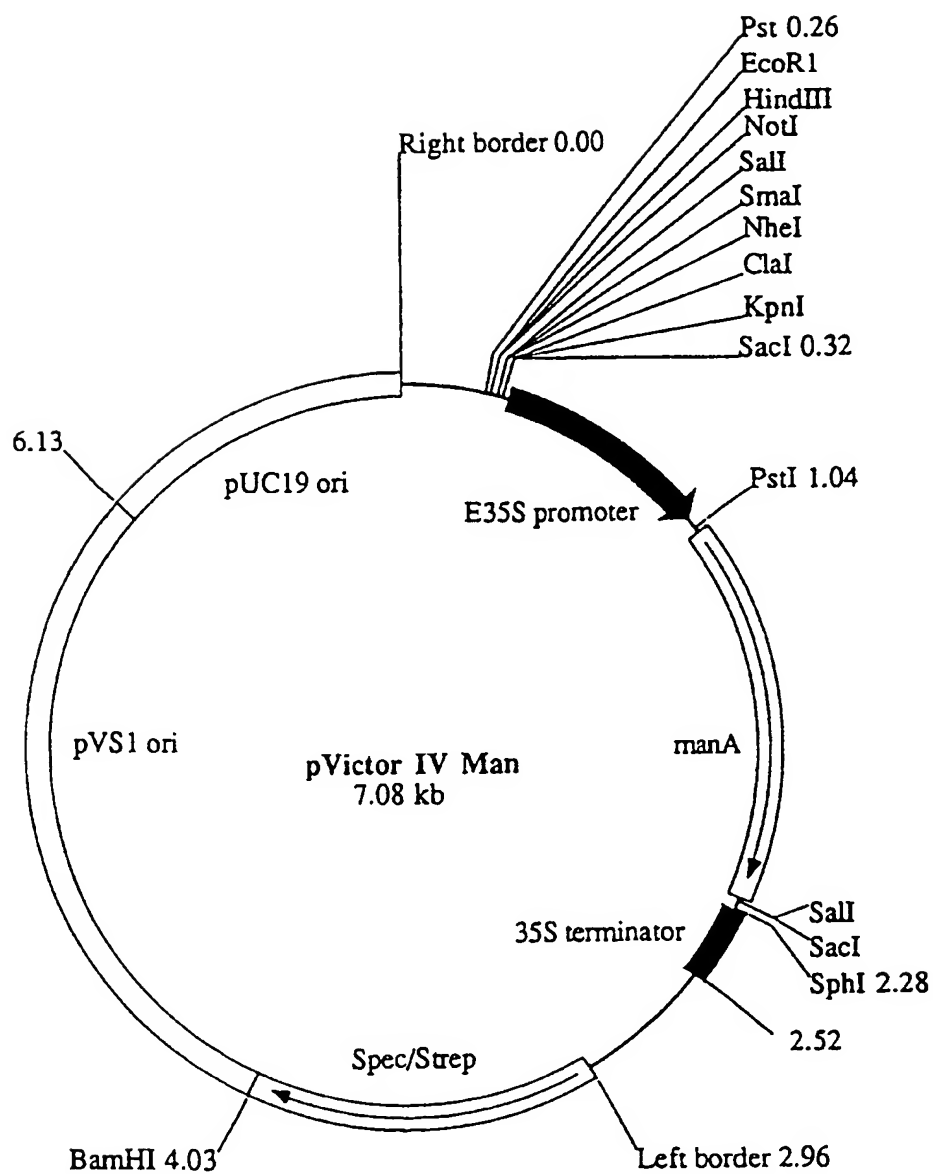


FIG. 7

8 / 27

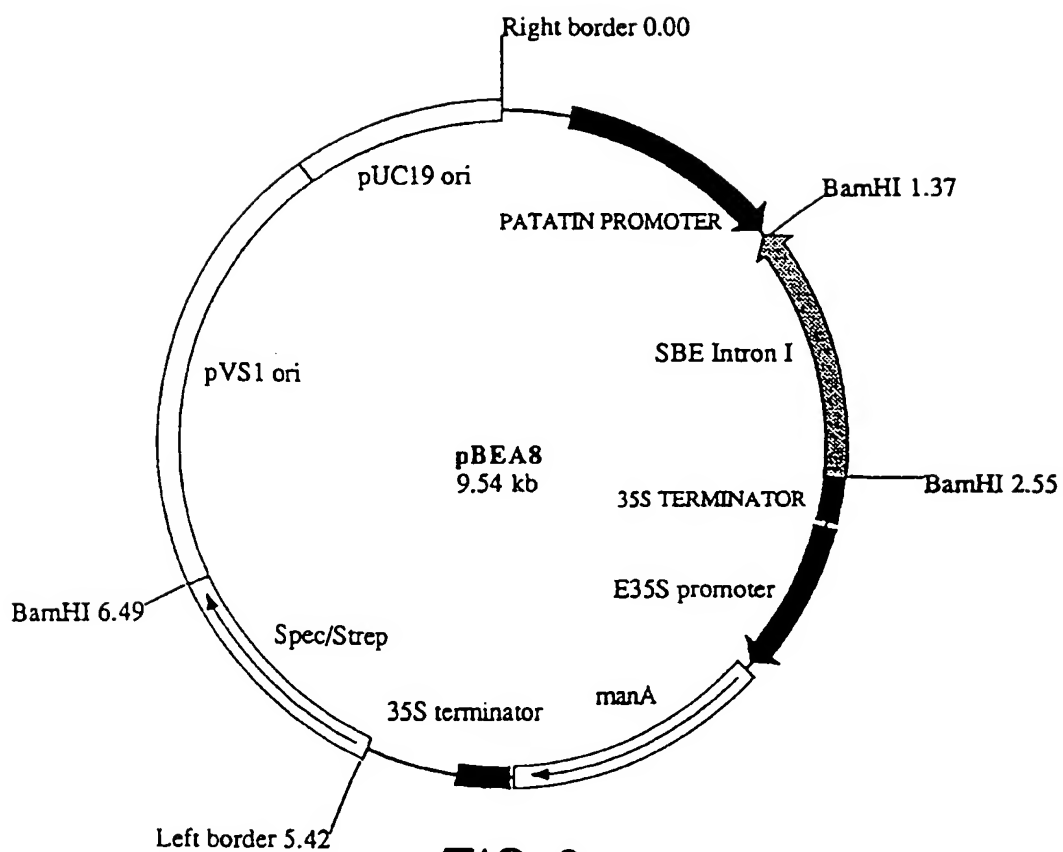


FIG. 8

9 / 27

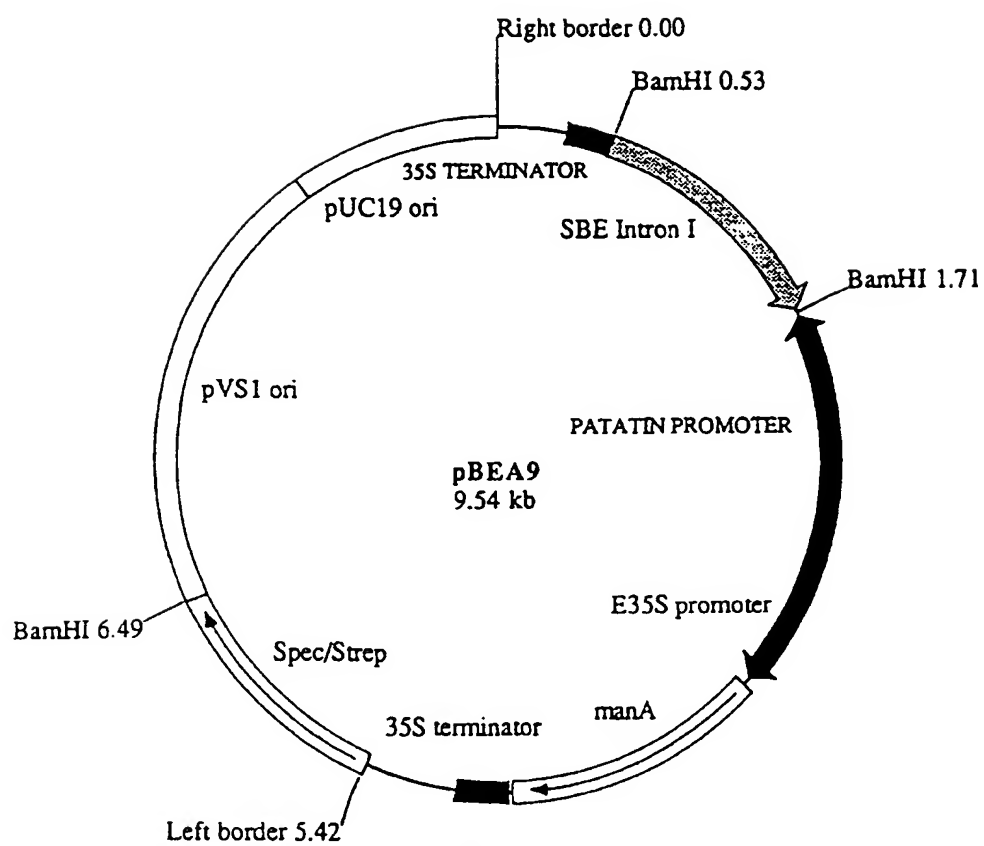


FIG. 9

10 / 27

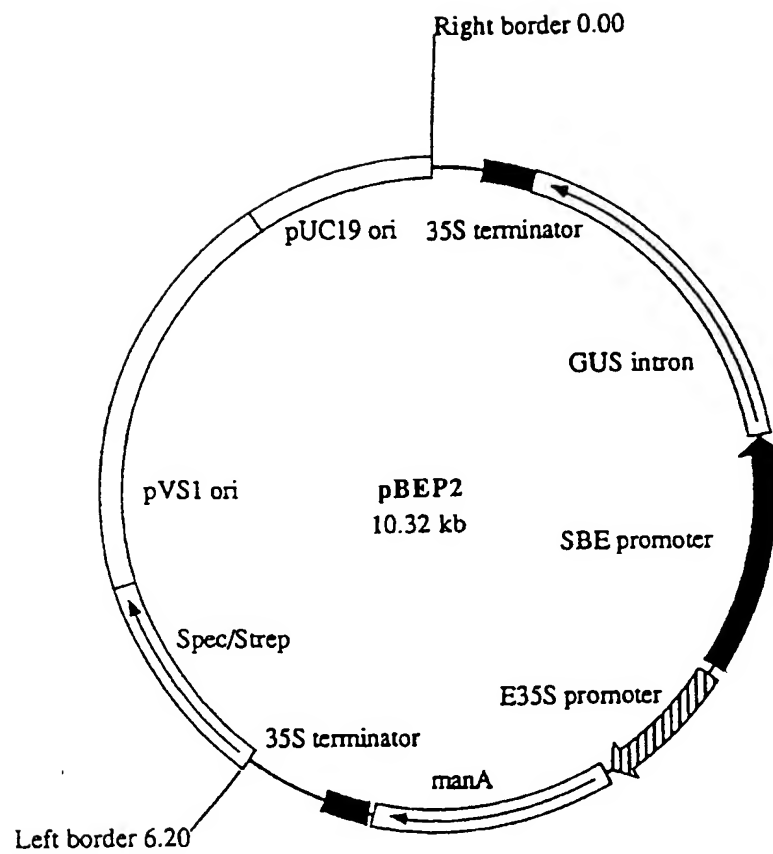


FIG. 10

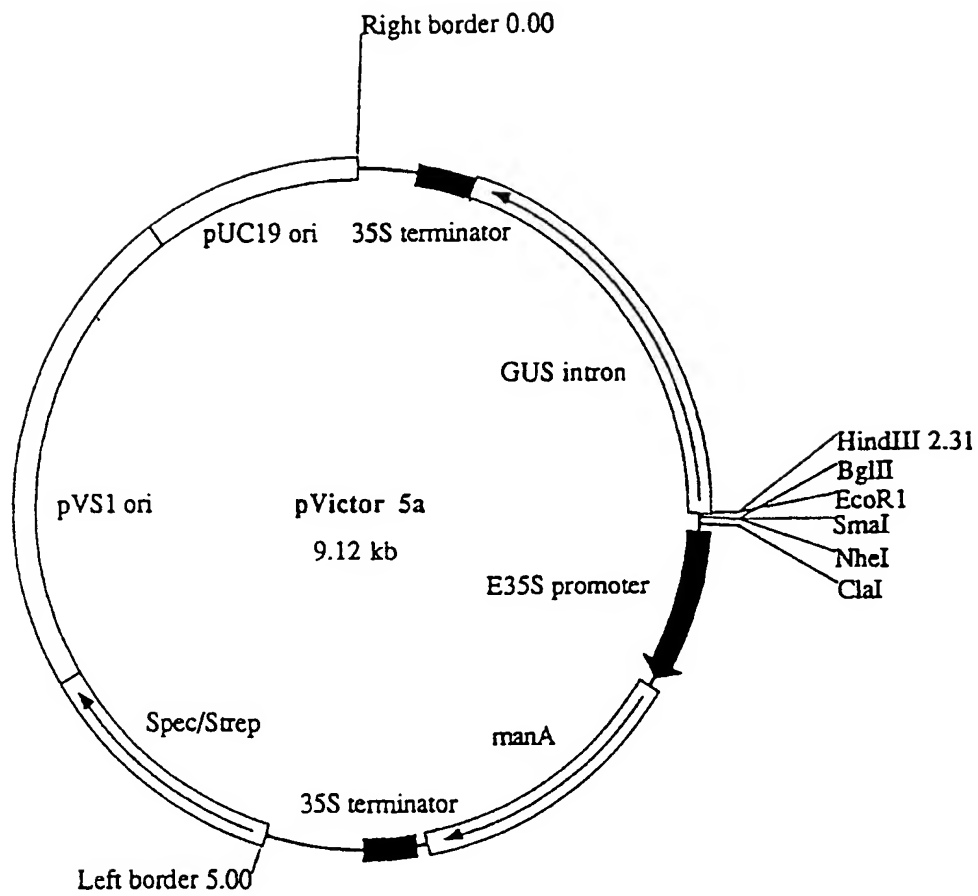


FIG. 11

12 / 27

10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
ATCATGGCCAATTACTGGTTCAAATGCATTACTTCCTTTCAGATTCTTTCGAGTTCTCAT						60
GACCGGTCCTACTACAGACGATACTAACCCGTGGAACGTGTCATCTGCTTCTTAGAACT						120
CTATGGCTATTTTCGTTAGCTTGGCGTCGGTTTGAACATAGTTTTTGTTCCTTCAAACCTCTT						180
CATTTACAGTCAAAATGTTGTATGGTTTTTGTTCCTCAATGATGTTTACAGTGTGTG						240
TTGTCATCTGTACTTTTGCTATTACTTGTTCGAGTTACATGTTAAAAAGTGTATT						300
TTGCCATATTTTGTCTCTTATTATTATTATCATACATACATTATTACAAGGAAAAGACA						360
AGTACACAGATCTTAACGTTTATGTTCAATCAACTTTTGGAGGCATTGACAGGTACCACA						420
AATTTTGAGTTTATGATTAAGTTCAATCTTAGAATATGAATTTAACATCTATTATAGATG						480
CATAAAATAGCTAATGATAGAACATTGACATTTGGCAGAGCTTAGGGTATGGTATATCC						540
AACGTTAATTTAGTAATTTTGTACGTACGTATATGAAATATTGAATTAATCACATGAA						600
CGGTGGATATTATATTATGAGTTGGCATCAGCAAAATCATTGGTGTAGTTGACTGTAGTT						660
GCAGATTTAATAATAAAATGGTAATTAACGGTCGATATTAAATAACTCTCATTTCAGT						720
GGGATTAGAACTAGTTATTAAAAAATGTATACTTTAAGTGATTTGATGGCATATAATTT						780
AAAGTTTTTCATTTCATGCTAAATTTGTTAATTATTGTAATGTAGACTGCGACTGGAATT						840
ATTATAGTGTAATTTATGCATTCAAGTGTAAATTAAGTATTGAACCTGTCTGTTTTAG						900
AAAATACTTTATACTTTAATATAGGATTTTGTATGCGAATTTAAATTAATCGATATGA						960
ACACGGAATACCAAAATTAAAAAGGATACACATGGCCTTCATATGAACCGTGAACCTTTG						1020
ATAACGTGGAAGTTCAAAGAAGGTAAAGTTTAAAGAATAAACTGACAAATTAATTTCTTTT						1080
ATTTGGCCCACTACTAAATTTGCTTTACTTTCTAACATGTCAAGTTGTGCCCTCTTAGTT						1140
GAATGATATTTCATTTTCATCCCATAGTTCAATTTGATTGTATACCACCCATGATGTT						1200
CTGAAAAATGCTTGGCCATTACAAAGTTTATCTTAGTTCCATGAACTTTATAAGAAGC						1260
TTTAATTTGACATGTTATTTATATTAGATGATATAATCCATGACCCAATAGACAAGTGTA						1320
TTAATATTGTAACTTTGTAAATGAGTGTGTCTACATCTTATTCAATCATTTAAGGTCATT						1380
AAAATAAATTATTTTTTGACATTCTAAACTTTAAGCAGAATAAATAGTTTATCAATTAT						1440
TAAAAACAAAAACGACTTATTTATAAATCAACAAACAATTTTAGATTGCTCCAACATAT						1500

FIG. 12

SUBSTITUTE SHEET (rule 26)

13 / 27

10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
TTTTCCAAATTAAATGCAGAAAATGCATAATTTTATACTTGATCTTTATAGCTTATTTT						1560
TTTAGCCTAACCAACGAATATTTGTAACTCACAACCTTGATTAAGGGATTACAACAA						1620
GATATATATAAGTAGTGACAAATCTTGATTTTAAATATTTTAATTTGGAGGTCAAAATTT						1680
TACCATAATCATTTGTATTTATAATTAATTTTAAATATCTTATTTATACATATCTAGTA						1740
AACTTTTAAATATACGTATATACAAAATATAAAATTTATGGCGTTCATATTAGGTCAATA						1800
AATCCTTAACATATCTGCCTTACCACTAGGAGAAAGTAAAAACTCTTTACCAAAAATA						1860
CATGTATTATGTATACAAAAGTCGATTAGATTACCTAAATAGAAATGTATAACGAGTA						1920
AGTAAGTAGAAATATAAAAAAACTACAATACTAAAAAAATATGTTTTACTTCAATTTTCG						1980
AAACTAATGGGGTCTGAGTGAAATATTCAGAAAGGGAGGACTAACAAAAGGTCATAAT						2040
GTTTTTTTATBAAAAGCCACTAAAATGAGGAAATCAAGAATCAGAACATACAAGAAGGCA						2100
GCAGCTGAAGCAAAGTACCATAATTTAATCAATGGAAATTAATTTCAAAGTTTATCAAAA						2160
			M E I N F K V L S K			
ACCCATTCGAGGATCTTTTCCATCTTTCTCACCTAAAGTTTCTTCAGGGtaatttttac						2220
P I R G S F P S F S P K V S S G						
taatttcattgtaattttcaattatttttagcctttgcatttcattttccaatatatctgg						2280
atcatctccttagttttttattttattttttataatatcaaatatggaagaaaaatgaca						2340
cttgtagagccatagtgaagtatcatgtgacaaatttgcaaggtggttgagtgtataaaa						2400
ttcaaaaattgagagatggagggggggtgggggbaragacaatattagaaagagtgttc						2460
taggaggttatggaggacacggatgaggggtagaagggttagttaggtatttgagtgttgt						2520
ctggccttatcctttcatactagtagtcgtggaattatttgggtagtttcttggtttgtta						2580
tttgatctttgttattctattttctgtttcttgacttcgattattgtattatatatctt						2640
gtcgtagttattgttcctcggaagaatgctctagcatgcttccttttagtggtttatcat						2700
gccttcttttatattcgcggttgctttgaaatgcttttacttttagccgagggctattagaa						2760
acaatctctctatctcgtaaggtaggggtaaaagtcctcaccacactccacttggtgggatt						2820
acattgtgtttgttgttgtaaatcaattatgtatacataataagtggtttttacaaca						2880
caaatacatgggtcaagggcaaagttctgaacacataaaaggggttcatttatgtccagggga						2940
tatgataaaaattgtttctttgtgaaagttatataagatttgttatggccttttgctggaa						3000

FIG. 12 CONTINUED

SUBSTITUTE SHEET (rule 26)

14 / 27

10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
acataataagttataatgctgagatagctactgaagtttgtttttctagccttttaaat						3060
gtaccaataatagattccgtatcgaacgagtatgttttgattacctggtcatgatgtttc						3120
tattttttacatttttttgggtgttgaactgcaattgaaaatgttgatcctatgagacgg						3180
atagttgagaatgtgttctttgtatggaccttgagaagctcaaacgctactccaataatt						3240
tctatgaattcaaattcagttttatggctaccagtcagtcagaaaattaggatagctgca						3300
tatacttgttcaattatactgtaaaattttcttaagttctcaagatatccatgtaacctcg						3360
agaatttctttgacagGCTTCTAGAAATAAGATATGTTTCCTTCTCAACATAGTACTGG						3420
ACTGAAGTTTGGATCTCAGGAACGGTCTTGGGATATTTCTTCCACCCCAAAATCAAGAGT						3480
L K F G S Q E R S W D I S S T P K S R V						
TAGAAAAGATGAAAGGgtatgtttgataatttatatggttgcatggatagtatataaata						3540
R K D E R						
gttggaataacttctggactggtgctcatggcatatgtgatctgtgcaccgtgtggagatg						3600
tcaaacatgtgttacttcgttccgccaatttataataccttaacttgggaagacagctc						3660
tttactcctgtgggcatttgttatttgaattacaatctttatgagcatggtgttttcaca						3720
ttatcaacttctttcatgtggtatataacagtttttagctccgttaatacctttcttctt						3780
tttgatataaaactaactgtggtgcatgtgcttgcbbkkaATGAAGCACAGTTCAGCTATTTT						3840
CGCTGTTTTGACCGATGACGACAATTTCGACAATGGCACCCCTAGAGGAAGATGTCAAGAC						3900
A V L T D D D N S T M A P L E E D V K T						
TGAAAATATTGGCCTCCTAAATTTGGATCCAACCTTTGGAACCTTATCTAGATCACTTCAG						3960
E N I G L L N L D P T L E P Y L D H F R						
ACACAGAATGAAGAGATATGTGGATCAGAAAATGCTCATTGAAAAATATGAGGGACCCCT						4020
H R M K R Y V D Q K M L I E K Y E G P L						
TGAGGAATTGCTCAAGgtaacagccaaaagttgtgcttttaggcagtttgaccttatttt						4080
E E F A Q G						
ggaagatgaattgtttatacctactttgactttgctagagaattttgcataccggggagt						4140
aagtagtggctccatttaggtggcacctggccatttttttgatcttttaaaaagctgttt						4200
gattgggtcttcaaaaaagtagacaaggtttttgagaagtgacacacccccggagtgtc						4260
agtggcaaaagcaaagattttcactaaggagattcaaaatataaaaaaagtatagacataa						4320
agaagctgaggggattcaacatgtactatacaagcatcaaatatagtcttaagcaattt						4380
tgtagaataaagaaagtccttcttctgttgcctcacaatttcttctattatcatgagt						4440
tactctttctgttcgaaatagcttccttaataattaaattcatgatacttttgttgagatt						4500

FIG. 12 CONTINUED

SUBSTITUTE SHEET (rule 26)

15 / 27

10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
tagcaggtttttcttctgtgtaaactgctctctttttttgagGTTATTTAAATTTGGATT						4560
				Y L K F G F		
CAACAGGGAAGATGGTTGCATAGTCTATCGTGAATGGGCTCCTGCTGCTCagtaggtccc						4620
N R E D G C I V Y R E W A P A A Q						
cgtctactacaaaatagtagtttccatcatcataacagattttcctattaaagcatgatg						4680
ttgcagcatcatgtggctttcttcatgttcttaattgctattaaaggttatgtcttctaatta						4740
actcatccacaatgcagGGAAGCAGAAGTTATTGGCGATTTCAATGGATGGAACGGTTCT						4800
				E A E V I G D F N G W N G S		
AACCACATGATGGAGAAGGACCAGTTTGGTGTGGAGTATTAGAATTCCTGATGTTGAC						4860
N H M M E K D Q F G V W S I R I P D V D						
AGTAAGCCAGTCATTCCACACAATCCAGAGTTAAGTTTCGTTTCAAACATGGTAATGGA						4920
S K P V I P H N S R V K F R F K H G N G						
GTGTGGGTAGATCGTATCCCTGCTTGGATAAAGTATGCCACTGCAGACGCCACAAAGTTT						4980
V W V D R I P A W I K Y A T A D A T K F						
GCAGCACCATATGATGGTGTCTACTGGGACCCACCACCTTCAGAAAGgttttgttattca						5040
A A P Y D G V Y W D P P P S E R						
taccttgaagctgaattttgaacaccatcatcacaggcatttcgattcatgttcttacta						5100
gtcttgttatgtaagacattttgaaatgcaaaagttaaataaattgtgtctttactaatt						5160
tggacttgatcccatactctttcccttaacaaaatgagtcatttctataagtgttgaga						5220
acttactactttcagcaattaaacagGTACCACTTCAAATACCCTCGCCCTCCCAAACCCC						5280
				Y H F K Y P R P P K P R		
GAGCCCCACGAATCTATGAAGCATGTGCGCATGAGCAGCTCTGAGCCACGTGTAAATT						5340
A P R I Y E A H V G M S S S E P R V N S						
CGTATCGTGAGTTTGCAGATGATGTTTACCTCGGATTAAGGCAAATAACTATAACTG						5400
Y R E F A D D V L P R I K A N N Y N T V						
TCCAGTTGATGGCCATAATGGAACATTCTTACTATGGATCATTGGATATCATGTTACAA						5460
Q L M A I M E H S Y Y G S F G Y H V T N						
ACTTTTTTGCTGTGAGCAGTAGATATGGAACCCGGAGGACCTAAAGTATCTGATAGATA						5520
F F A V S S R Y G N P E D L K Y L I D K						
AAGCACATAGCTTGGGTTTACAGGTTCTGGTGGATGTAGTTTCACAGTCATGCAAGCAATA						5580
A H S L G L Q V L V D V V H S H A S N N						
ATGTCACTGATGGCCTCAATGGCTTTGATATTGGCCAAGGTTCTCAAGAATCTACTTTC						5640
V T D G L N G F D I G Q G S Q E S Y F H						
ATGCTGGAGAGCGAGGTACCATAAGTTGTGGGATAGCAGGCTGTCAACTATGCCAATT						5700
A G E R G Y H K L W D S R L F N Y A N W						
GGGAGGTTCTTCGTTTCCTTCTTCCAACCTGAGGTGGTGGCTAGAAGAGTATAACTTTG						5760
E V L R F L L S N L R W W L E E Y N F D						
ACGGATTTGATTTGATGGAATAACTTCTATGCTGTATGTTTCATCATGGAATCAATATGG						5820
G F R F D G I T S M L Y V H H G I N M G						
GATTTACAGGAACTATAATGAGTATTTTCAGCGAGGCTACAGATGTTGATGCTGTGGTCT						5880
F T G N Y N E Y F S E A T D V D A V V Y						
ATTTAATGTTGGCCAATAATCTGATTACAAGATTTTCCAGATGCAACTGTTATTGCCG						5940
L M L A N N L I H K I F P D A T V I A E						
AAGATGTTTCTGGTATGCCGGCCCTTGGCCGGCCTGTTTCTGAGGGAGGAATTGGTTTTC						6000
D V S G M P G L G R P V S E G G I G F V						

FIG. 12 CONTINUED

SUBSTITUTE SHEET (rule 26)

16 / 27

10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
TTTACCGCCTGGCAATGGCAATCCCAGATAAGTGGATAGATTATTTAAAGAATAAGAATG						6060
Y R L A M A I P D K W I D Y L K N K N D						
ATGAAGATTGGTCCATGAAGGAAGTAACATCGAGTTTGACAAATAGGAGATATACAGAGA						6120
E D W S M K E V T S S L T N R R Y T E K						
AGTGTATAGCATATGCGGAGACCCATGATCAGGtatttttaattttatttctacaactaaa						6180
C I A Y A E T H D Q						
taattcttcagaacaattgttagatagaatccaaatatatacgtcctgaaagtataaaagt						6240
acttatttttcgccatgggccttcagaatatttggtagccgctgaatatcatgataagttat						6300
ttatccagtgacatttttatgttcactcctattatgtctgctggatacagTCTATTGTTG						6360
				S I V G		
GTGACAAGACCATTGCATTTCTCCTAATGGACAAAGAGATGTATTCTGGCATGCTCTGCT						6420
D K T I A F L L M D K E M Y S G M S C L						
TGACAGATGCTTCTCCTGTTGTTGATCGAGGAATTGCGCTTCACAAGgtttgtctgtttc						6480
T D A S P V V D R G I A L H K						
tattgcatttttaagggttcatatagggttagccacggaaaatctcactcttttgtgaggtaac						6540
cagggttctgatggattattcaattttctcgtttatcatcttgtttattcttttcatgcat						6600
tgtgttttctttttcaatatccctcttatttggaggtaatttttctcatctattcactttt						6660
agcttctaaccacagATGATCCATTTTTCACAATGGCCTTGGGAGGAGAGGGGTACCTC						6720
M I H F F T M A L G G E G Y L						
AATTTCATGGGTAACGAGgtatgtcttacatcttttagatattttgtgataattacaatta						6780
N F M G N E						
gtttggcttacttgaacaagattcattcctcaaaatgacctgaactgttgaaatcaaag						6840
gggttgaaacatagaggaaaacaacatgatgaatgtttccattgtctagggtatttctatt						6900
atgttgctgagaacaaatgtcatcttaaaaaaacattgtttactttttttagtataga						6960
agattactgtatagagtttgcaagtgtgtctgttttggagtaattgtgaaatgtttgatg						7020
aacttgtagagTTTGGCCATCCTGAGTGGATTGACTTCCCTAGAGAGGGCAATAATTGGA						7080
F G H P E W I D F P R E G N N W S						
GTTATGACAAATGTAGACGCCAGTGGAACTCGCGGATAGCGAACAATTGAGATACAAGg						7140
Y D K C R R Q W N L A D S E H L R Y K						
ttcaagtattttgaatcgagcttgttaataatcttagtaatttttagattgcttacttg						7200
gaagtctacttgggttctggggatgatagctcatttcatcttgttctacttattttccaac						7260
cgaattttctgatttttgtttcgagatccaagtatttagattcatttacacttattaccgcc						7320
tcatttctaccactaaggccttgatgagcagcttaagttgattcttttgaagctatagttt						7380
caggctaccaatccacagcctgctatatattgttggatacttaccttttctttacaatgaa						7440
gtgatactaattgaaatgggtctaaatctgatcttatatttctccgtcttctctccccct						7500

FIG. 12 CONTINUED

SUBSTITUTE SHEET (rule 26)

17 / 27

10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
catgatgaaatgcagTTTATGAATGCATTTGATAGAGCTATGAATTCGCTCGATGAAAAG						7560
	F M N A F D R A M N S L D E K					
TTCTCATTCTCGCATCAGGAAAACAGATAGTAAGCAGCATGGATGATGATAATAAGgta						7620
F S F L A S G K Q I V S S M D D D N K						
aaatcatctaaagtggaaagtgttgggtttatgaagtgccttaattctatccaaggacaa						7680
gtagaaacctttttaccttccatttcttgatgatggatttcatattatttaatccaatag						7740
ctggtcaaattcggtaatatgctgtactgattagttacttcactttgcagGTTGTTGTGTT						7800
	V V V F					
TGAACGTGGTGACCTGGTATTGTATTCAACTTCCACCCAAAGAACACATACGAAGGgta						7860
E R G D L V F V F N F H P K N T Y E G						
tatatgttttacttatccatgaaattattgctctgcttgttttaattgtactgaacaagt						7920
tttatggagaagtaactgaaacaaatcattttccattgtctaatttaactcttttttct						7980
gatcctcgcatgacgaaaacagGTATAAAGTTGGATGTGACTTGCCAGGGAAGTACAGAG						8040
	Y K V G C D L P G K Y R V					
TTGCACTGGACAGTGATGCTTGGGAATTGGTGGCCATGGAAGgtaaggatttgcttga						8100
A L D S D A W E F G G H G R						
ataacttttgataataagataacagatgtagggtacagttctctcaccaaaaagaactgt						8160
aattgtctcatccatcttttagttgtataagatatccgactgtctgagttcgggaagtgttt						8220
gagcctcctgccctccccctgcgttgttttagctaattcaaaaaggagaaaactgtttatt						8280
gatgatctttgtcttcatgctgacatacaatctgtttctcatgacagACTGGTCATGATGT						8340
	T G H D V					
TGACCATTTCACATCACCAGAAGGAATACCTGGAGTTCAGAAACAAATTTCAATGGTCG						8400
D H F T S P E G I P G V P E T N F N G R						
TCCAAATTCCTTCAAAGTGCTGTCTCTCGCGAACATGTGTGgtacagttcttgccgtg						8460
P N S F K V L S P A R T C V						
tgacctccctttttattgtgggtttgttcatagttatttgaatgcatagaagttaacta						8520
ttgattaccgccacaatcgccagtttaagtcctctgaactactaatttgaaaggtaggaat						8580
agccgtaataagggtctacttttggcatcttactgttacaaaacaaaaggatgccaaaaaa						8640
attcttctctatcctcttttttccctaaaccagtgcatgtagcttgccacctgcataaactt						8700
aggtaaatgatcaaaaatgaagttgatgggaacttaaaaccgccctgaagtaaaagctagg						8760
aatagtcataataatgtccacctttgggtgtctgctgaacatcaacaacaacatacctcgt						8820
gtagtcacacaaagtgggtttcagggggagggtagagtgtatgcaaaacttactcctatct						8880
cagaggtagagaggattttttcaatagacccttggtctcaagaaaaaaagtcacaaaagaa						8940
gtaacagaagtgaagcaacatgtgtagctaaagcgacccaacttgtttgggactgaagt						9000

FIG. 12 CONTINUED

SUBSTITUTE SHEET (rule 26)

18 / 27

10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
agttgttgttgttgaacagtgcatgtagatgaacacatgtcagaaatggacaacacag						9060
ttatrtttgtgcaagtcaaaaaaatgtactactatrttctttgtgcagctttatgtatagaa						9120
aagttaaataactaatgaatrttctgtagcagaaaaatagcttgagagaaaatrtttrata						9180
ttgaactaagctaactatattcatctrttctrttctgcttcttcttctccttgttgtgaag						9240
GCTTATTACAGAGTTGATGAACGCATGTCAGAACTGAAGATTACCAGACAGACATTTGT						9300
A Y Y R V D E R M S E T E D Y Q T D I C						
AGTGAGCTACTACCAACAGCCAATATCGAGGAGAGTGACGAGAACTTAAAGATTTCGTTA						9360
S E L L P T A N I E E S D E K L K D S L						
TCTACAAATATCAGTAACATTGACGAACGCATGTCAGAACTGAAGTTTACCAGACAGAC						9420
S T N I S N I D E R M S E T E V Y Q T D						
ATTTCTAGTGAGCTACTACCAACAGCCAATATTGAGGAGAGTGACGAGAACTTAAAGAT						9480
I S S E L L P T A N I E E S D E K L K D						
TCGTTATCTACAAATATCAGTAACATTGATCAGACTGTTGTAGTTTCTGTTGAGGAGAGA						9540
S L S T N I S N I D Q T V V V S V E E R						
GACAAGGAACTTAAAGATTCCCGTCTGTAAGCATCATTAGTGATGTTGTTCCAGCTGAA						9600
D K E L K D S P S V S I I S D V V P A E						
TGGGATGATTCAGATGCAAACGCTCGGGGTGAGGACTAGTCAGATGATTGATCGACCTT						9660
W D D S D A N V W G E D						
CTACCGATTGGTGATCGCTATCCTTGCTCTCTGAGAAATAGGTGAGGCGAAACAAAAAAT						9720
AATTTGCATGATAAAAAGTCTGATTTTATGATCGCTATCCTCGCTCTCTGAGAAAGAAGC						9780
GAAACAAAGGCGACTCCTGGACTCGAATCTATAAGATAACAAAGGCGACTCCTGGGACTC						9840
GAATCTATAAGATAACAAAGGCAATTCGAAGACTTGAATCTATAAAAAATTTAGTTAAGA						9900
ATGATTAACGTCCGATCCTAATTCGAATCGAGGCATCTTACCACTCCATTGATAATTATA						9960
TAAGTCAATAAGTCATATAAWAGTATTA AAAA ACTAAATTGACTTGATCGGTCTATCAAAA						10020
ATMAGATMAAATTGTGTTTCATATGTAACATTTTTGTTGTCACAATTAGCTTAATTACATC						10080
TTTCATGTGCAATAACAAAGAAATGATAGGAATTTAGAGATTCCAATTTTTTTGTTGCCA						10140
CAATTAACCTTAATTACATCTTTTCATTTGCAATAACAAAGAAATGATAGGAATTTAGAGAT						10200
CCAGTGTCAAATACACAACCTAGGCCAACATCGAAAGCATAACTGTAACTCATGCATGAA						10260
GAAATCAGTCGTAAAAATGAATAAATGCGACATAAAAACAAATTGCATGTATCATTAAATG						10320
TGACTTAACTACAAGTAAAAATAAATTTAACAAATGTAACCTTAACTACAAGTAAAAATAA						10380
ATTGCTTCTATCATTAACAAACAAACAGAAATTA AAAA AGAAAAAACATACTAAATCTTAC						10440
CGTCATTGATAAAAAAATAACCAAATTCATAATGCAAGGAAACGAAACGCGTCCTGA						10500

FIG. 12 CONTINUED

SUBSTITUTE SHEET (rule 26)

19 / 27

10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
TCGGGTATCAACGATGAAATGGACCAGTTGGATCGACTGCCTGCACAACGTTAGGTATGC						10560
CAAAAAAAGAACACGATCCTTTGCACCCGTTTCGATGATTATCAGTATGTTACAAAAAA						10620
AACTTAAGTTCATCCAGTGTACAACAGCCCCAACATCTGCCCCCAAGTAACAAAAACAA						10680
CCAATTTATCTTATCTTATCTGCCACAAAATAATCGGTTTCACACTATCTCTTGTAT						10740
ACAAAATGACAAGTAGGAAGGAGAGGAGTCATCCAAATAAACGGTGCACGTTCTTTGAG						10800
AAAAGTCTTATTTTCGTAAGATCCAATTTCAACAACTTTTCTTCAAGTCAAAATTCCT						10860
GATAGTGTATCTCCTCTCGACGACCTCTTGCAATTGAACGATCTCCGCTTATCATGAAAAG						10920
TTGCTTGGATAACAAGTATTGCAAGGGGGGACAGTAGCTATTAAGTTAGTCGGCCCAAG						10980
GAAATGGAGGAGTGATAGTCTCGAATATTATTACCTCTTTAGCATTACCCGGTCTGGCT						11040
TTAAGGAGTTACGTCTTTTACGCTCGCCAATTTCTTTTTTTAGAATGGTTGGTGCAAAA						11100
TCGCGAGTTGTGGAAGGTTCAAGTTACTCGATTCTGTGATTTTCAAGTATGAGTGGTGAGA						11160
GAGATTCGATATTTTACGAGGTGTATTTCGAGGTCTAGTAGAACGAAGGTTGCACTAAT						11220
GAAAGTTTCAAGAGTTCATCATCATCTTCTTCTAGTAGATTTTCGCTTTCAAATGAGTAT						11280
GAAAATTCCTCCTCTTTTCTATTGATTTTCTTCATTGTTTTCTTCATTGTTGTGGTTGTT						11340
ATTGAAAAGAAAAGAAAATTTATAACAGAAAAAGATGTCAAAAAAAGGTAAATGAAAGA						11400
GTATCATATACTTAAAGAGTTGCGTAGAGATAAGTCAAAAGAAACAGAATTATAGTAATT						11460
TCAGCTAAGTTAGAATTC						11478

FIG. 12 CONTINUED

20 / 27

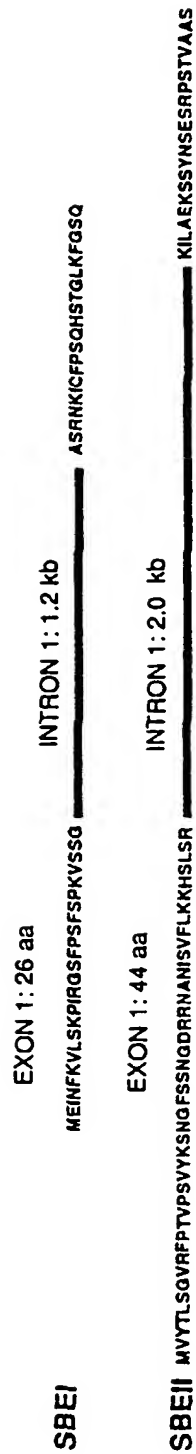


FIG. 13

21 / 27

10	20	30	40	50	60
12345678901234567890123456789012345678901234567890					
<hr/>					
GTATACACTCTCTGGAGTTCGTTTTCTACTGTTCCATCAGTGTACAAATCTAATGGATT					
Y T L S G V R F P T V P S V Y K S N G F					
SspI					
BsmI					
CAGCAGTAATGGTGATCGGAGGAATGCTAATATTTCTGTATTCTTGAAAAACACTCTCT					
S S N G D R R N A N I S V F L K K H S L					
BsaAI					
TTCACgtatgtctcactgtgtttgtggctgtgtgtgttttttctctgtctttttgtgtt					
S R					
Bsp1286I					
BanII					
ttgtgtaattggggctctttaagttggtattgtgtatacccttttgagtatagtctttg					
aggaagcaaaatgatgaatcttgattgacattagtaagggttgtaactttttgaagtttg					
gttaggtgtaattgagtttggtctgtgtctgtgtgtcgaggttattttttggtttgt					
gttattggggatcttaaaagttggtattgtgtatacccttttgagtatagtctttgagga					
agcaaaaatgatgaatcttgattggcattagtaaagggtttagctttttgaagtgtggtt					
agggtgaattgagtttggtctgtgtctgtgtgttttggaaatcctgatgtgtgtcaagt					

FIG. 14

SUBSTITUTE SHEET (rule 26)

22 / 27

10	20	30	40	50	60
123456789012345678901234567890123456789012345678901234567890					
<hr/>					
cctgatatgggtcgaggttctttctttggtttgtgtaattgggggttctttaaagtgtgt					
600					
attatgtacctttttaagaatagtgtctgagaaagcaaaatcgatgaattttgattgaca					
660					
gcatattctttgagaaagcaaaaaatggtgagttttcatggagaaacttgattgacatta					
720					
ctaaaggtagcaactttttcaactcctgatatgggtcaaggttctttggtttggtgtgt					
780					
aatttgggggttctttgaagttttgagaaagaaaaattatgatttttcatggagaaatttg					
840					
atttacattaataaaggtagtagctttttaagtggtcagctgtaatgagttcagctt					
900					
ggtttaaaggggcccctacatatggtgctttctggtgagatattgttgctccaccatac					
960					
gagttataagaatcatagtgttaggatctttttcttttttttttcattttccacttgac					
1020					
tagctactagaggagtgatcttgacggcgggaaaatcttagaaaggggaaggttgtttgca					
1080					

FIG. 14 CONTINUED

SUBSTITUTE SHEET (rule 26)

10	20	30	40	50	60
123456789012345678901234567890123456789012345678901234567890					

SUBSTITUTE SHEET (rule 26)

24 / 27

10	20	30	40	50	60
123456789012345678901234567890123456789012345678901234567890					

tccctgaaggaatagtttggttgatattgattatttcttggtttgtttaattcgggtgttc 1680

ttgaaggccatttttaaaccctttgacattgttaaagggtgtttacaagtgtgggtctgggt 1740

ttaaaagcacctcttgatgggtgctttctggagtgatctttcttctccaaaagagaagt 1800

BclI BglII

▼ ▼

tgcaagaatcagtggtgtacttttttctcttgatgatcagatcttttttcaatttttc 1860

cgtttttagttgatttatccatatagtgaaggtgggtgcatagttgctgtttgtggactt 1920

cctgtaaaagttttttgatatacttaaaaaattgtcacacagaagaagagttttttacc 1980

AflII

▼

attacttaagctagatgggactgtttgattcttagaccaaataatgaacctttttgttct 2040

AflIII

▼

cttaacgtgtacttgaaatagtttggtaaaattgtgataggaaaaagataattcttgat 2100

EaRI

▼

tgcttttgagcatcacttctaatacataaaagtctttgctctcttcaaccatgaatgata 2160

FIG. 14 CONTINUED

SUBSTITUTE SHEET (rule 26)

aattggacacttatgtggccctaagttgctctcagtagtggctcttaattgtggagatat 2220

aactaatctgatatatgtatgtagCGAAGATCTTGGCTGAAAGTCTTCTTACAATTCGG 2280
K I L A E K S S Y N S E

Sfci

AATCCCGACCTTCTACAGTTGCAGCATCG 2309

S R P S T V A A S

FIG. 14 CONTINUED

26 / 27

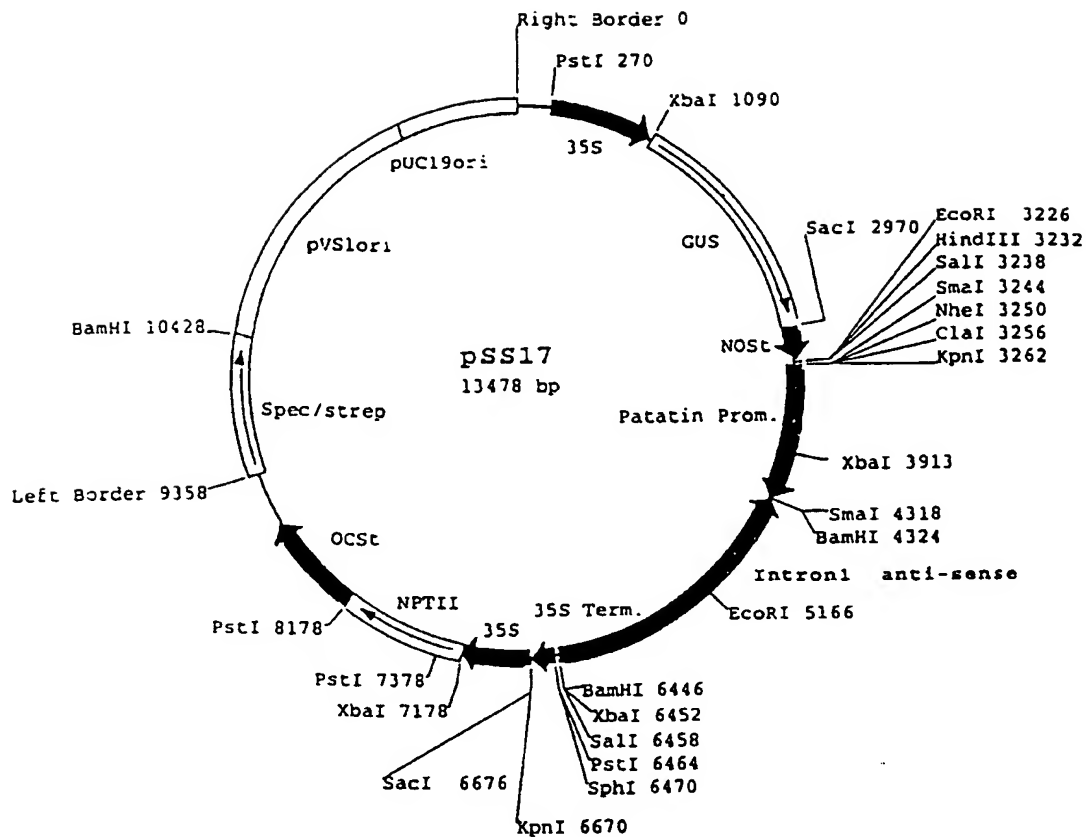


FIG. 15

27 / 27

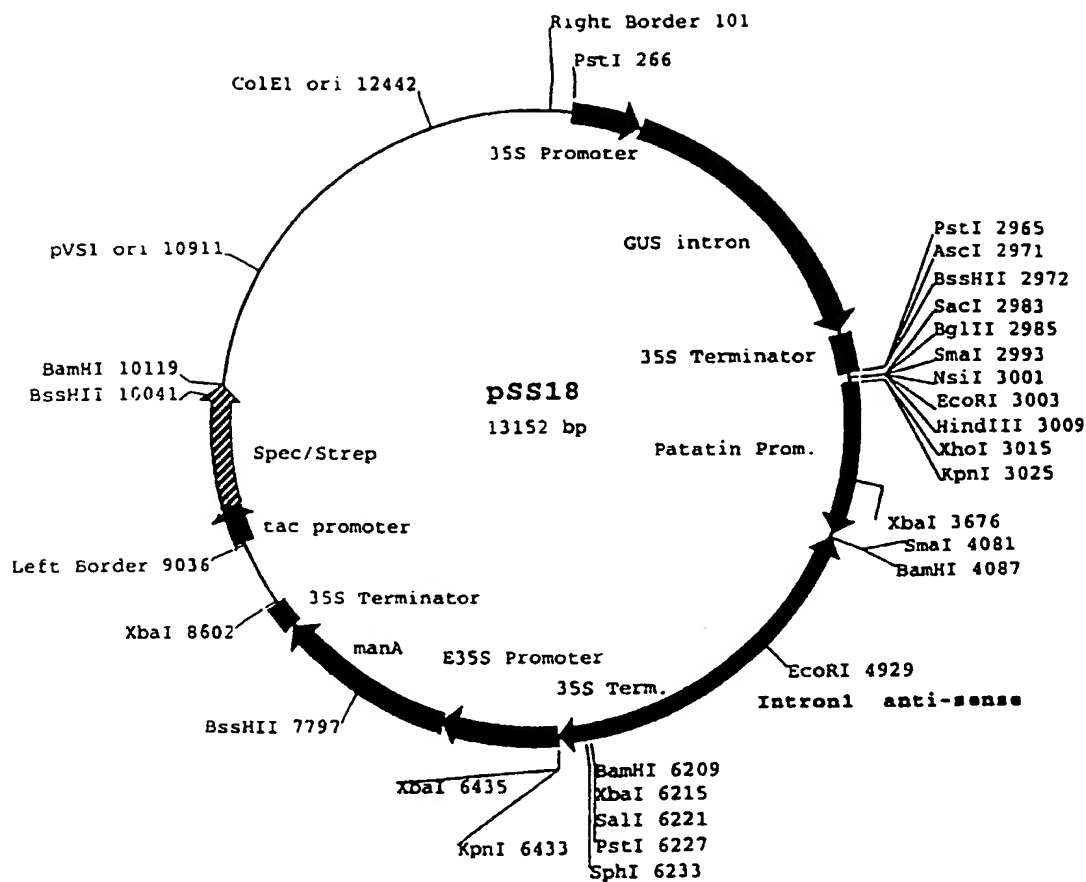


FIG. 16

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 98/00270

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/82 C12N9/10 C12N15/11 C08B30/04		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C12N C08B		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	WO 97 04112 A (DANISCO ;POULSEN PETER (DK)) 6 February 1997 cited in the application see the whole document ---	1-21
X	WO 97 04113 A (DANISCO ;POULSEN PETER (DK)) 6 February 1997 cited in the application see the whole document ---	1-21
Y	WO 96 34968 A (NAT STARCH CHEM INVEST ;COOKE DAVID (GB); DEBET MARTINE (GB); GIDL) 7 November 1996 cited in the application see page 5, paragraph 3 - paragraph 4 see page 9, paragraph 2 - page 10, paragraph 1	1-21
X	see page 11, paragraph 3 --- -/--	17-19
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 29 May 1998		Date of mailing of the international search report 09/06/1998
Name and mailing address of the ISA European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Tx 31 651 epo nl, Fax (+31-70) 340-3016		Authorized officer Chakravarty, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 98/00270

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	WO 92 11375 A (AMYLOGENE HB) 9 July 1992 cited in the application see the whole document ---	1-21
Y	WO 94 09144 A (ZENECA LTD) 28 April 1994 see page 10, line 1 - line 18 ---	1-21
Y	WO 92 15680 A (UNIV TEXAS) 17 September 1992 see page 6, line 17 - line 28 ---	1-21
X	EP 0 240 208 A (CALGENE INC) 7 October 1987 see page 3, line 10 - line 13 -----	15

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte: Jnal Application No

PCT/IB 98/00270

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9704112 A	06-02-1997	AU 6614596 A EP 0839202 A	18-02-1997 06-05-1998
WO 9704113 A	06-02-1997	AU 6614696 A EP 0839203 A	18-02-1997 06-05-1998
WO 9634968 A	07-11-1996	AU 5509996 A EP 0826061 A	21-11-1996 04-03-1998
WO 9211375 A	09-07-1992	SE 467160 B AU 9109791 A EP 0563201 A PL 169859 B SE 9004095 A	01-06-1992 22-07-1992 06-10-1993 30-09-1996 01-06-1992
WO 9409144 A	28-04-1994	CA 2146998 A AU 690517 B AU 2696492 A EP 0664835 A	28-04-1994 30-04-1998 09-05-1994 02-08-1995
WO 9215680 A	17-09-1992	AU 663702 B AU 1570492 A CA 2108144 A EP 0575518 A US 5747469 A	19-10-1995 06-10-1992 07-09-1992 29-12-1993 05-05-1998
EP 0240208 A	07-10-1987	AT 114168 T AU 1301792 A AU 618234 B AU 7059787 A DE 3750755 D DE 3750755 T EP 0458367 A ES 2066759 T JP 2702921 B JP 62296880 A JP 10052283 A US 5107065 A US 5453566 A US 4801540 A	15-12-1994 03-09-1992 19-12-1991 01-10-1987 22-12-1994 18-05-1995 27-11-1991 16-03-1995 26-01-1998 24-12-1987 24-02-1998 21-04-1992 26-09-1995 31-01-1989